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ERRATA

VOLUME 3, NUMBER 5, SEPTEMBER, 1933

Page 370: Legend to figure 1 should read "Lung Opened to Show Lesions."
Page 371: Legend to figure 2 should read "Section of Lung Showing Monilia (Gram's stain, oil immersion)."

THE PHOTELOMETER* AND ITS USE IN THE CLINICAL LABORATORY†

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PHOTO-ELECTRIC HEMOGLOBINOMETRY

In previous communications[5, 6, 7] we have presented the results of our investigations regarding the use of the photo-electric cell as an optico-electric device for the quantitation of hemoglobin, employing either a suitable galvanometer or one stage of amplification with a microammeter as the instrument for measuring the current produced by light incident on the photo-sensitive cell after previous transmission through a selective absorption filter and a specified dilution of blood. Experimental data were obtained by the methods of Van Slyke,[4, 8] spectrophotometer[1] and photo-electric hemoglobinometer. The data obtained substantiated the belief that the photo-electric hemoglobinometer, when used in connection with a selective filter transmitting light only in the region of the beta absorption band of oxyhemoglobin, will permit of the determination of grams of hemoglobin for each 100 c.c. of blood within an average of 2 per cent of the Van Slyke values, which have been taken as standards.

* The Sheard and Sanford photelometer will be manufactured by the Central Scientific Company of Chicago, Illinois. Any patent rights granted to the inventors, as well as any royalties accruing to them from the manufacture and sale of the photelometer, will be assigned to the American Society of Clinical Pathologists. These applications for patent rights have been made in order to control the development, accuracy and serviceability of such instruments, so that those who acquire apparatus involving the principles which have been or are to be disclosed may secure satisfactory equipment.

† Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

Photo-electrometers with one stage of amplification, made according to the disclosures given in these papers, have been operated very satisfactorily at The Mayo Clinic for three to four years in both clinical and experimental investigations concerning hemoglobin. During this period more than 200,000 determinations of grams of hemoglobin per 100 c.c. of blood have been made.

In the last year we have extended our researches along two lines: (1) the development of an improved instrument, known as the photelometer, employing a photronic cell and a constant source of illumination operating on the ordinary 110–115 volt alternating current circuits, and (2) the application of photo-electrometry to various types of solution and of various substances in solution which are chiefly of significance in clinical laboratories.

FUNDAMENTAL PRINCIPLES OF PHOTELOMETRY (PHOTO-ELECTRIC PHOTOMETRY)

In order to avoid repetition of details concerning the fundamental principles of photo-electric photometry, reference should be made to the previously published communications which have been cited in the foregoing paragraphs. By way of generalized statment, however, it may be well to point out that the light-sensitive cell (whether it be photo-conductive, photo-electric or photo-voltaic) is an electrical eye which does not suffer from the elements of fatigue or uncertainty in the determination of the amount or equality of illumination (as obtained by methods involving matches of equality of illumination in colorimetry) as in the case of the eye of man. In general, the current which flows in the circuit containing the photo-sensitive cell is proportional to the illumination which the cell receives. If the photo-sensitive cell is illuminated by a source of light which can be kept constant, the reading on a sufficiently sensitive instrument (such as a micro-ammeter) will be a constant. When, therefore, a solution of a substance which contains a characteristic absorption band is placed between the source of illumination and the photo-sensitive cell, together with a selective spectral filter (which may be of special glasses, dyed gelatin films or appropriate chemicals in solution) which transmits light only in the region of the char-

acteristic band, the amount of light which reaches the photo-sensitive cell will depend on the concentration of the substance under test which possesses the specified absorption band. According to the laws of Lambert and Beer, the concentration C of a given substance will be directly proportional to the negative logarithm of the unabsorbed light I. Since the current A in the circuit containing the photo-sensitive cell is directly proportional to the illumination I which it receives, it follows that the concentration of the substance under investigation which possesses a characteristic absorption zone when in solution is directly proportional to the negative logarithm of the unabsorbed light or, in other words, to the negative logarithm of the transmitted light. Hence, if C_1 and C_2 represent the concentrations of two given solutions of the same substance, I_1 and I_2 the amounts of transmitted light and A_1 and A_2 the readings of the current on the measuring instrument, then

$$\frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2} = \frac{-\log A_1}{-\log A_2}$$

REASONS FOR DEPARTURE OF PRACTICAL PHOTELOMETRY FROM THEORY

The ideal conditions which we have just outlined are met but rarely in actual practice. Among the reasons which may be cited to account for these deviations are: (1) Difficulty in obtaining filters which are feasible for ordinary technical routine and which have transmission bands corresponding exactly to the characteristic absorption band or zone selected. The curve of transmission of the filter should be the mirror image of the curve of absorption. (2) Variability in or change of shape of absorption band, and therefore the values of the absorption of light for the various wavelengths involved, with degree of concentration of the material in solution. Strictly speaking, the law $C = -k \log I$ holds only under the condition that the measurements of the unabsorbed light are made at a specified wavelength, such as that corresponding to the maximal absorption of light. (3) Lack of linearity or proportionality in the relationship between intensity of illumination I and the current A developed.

However, in spite of these factors which cause departure from the theoretically ideal situation, as well as other factors which perchance should be cited, photo-electric photometry furnishes a rapid and accurate method for the determination of the concentrations of various substances in solution under as close an observance, as is practically possible, of those criteria which we have pointed out. An approximation to those criteria or conditions imposed for the highest accuracy possible permits the operator to obtain a curve (in general, to be classed as an exponential curve) showing the relationship between concentration and readings on the current-measuring instrument. Such data, in general, when plotted on semi-logarithmic paper, will not give a straight line throughout the course of the plotted curve, although in many cases such plots will disclose the fact that, for a considerable range of concentrations and readings of current, the logarithmic law of Lambert and Beer is obeyed within a small percentage of error. When such is the case, the operator may dilute the solutions in such definite quantities as to cause the readings of the current to fall on the straight line portion of the plot and thereby insure the greatest accuracy possible under the method. Or, on the other hand, the operator may not concern himself about these matters but may proceed to obtain a curve showing the relationship between concentration and readings of the current (such as curve 1, figure 3) using a spectral filter which corresponds as closely as possible to an absorption band or zone of the substance under measurement. From such a curve, he may make a tabulation of corresponding concentrations and current readings. Such a procedure doubtless would be the best routine practice in the clinical laboratory, since a reading of the current would be transferred immediately from the table to the corresponding value of the concentration of the material in solution.

DIAGRAMMATIC SKETCH OF THE MODUS OPERANDI OF THE PHOTELOMETER

Figure 1 illustrates the essential features and, in general, the modus operandi of the photelometer. A constant source of light

is operated from a specially designed transformer in order that the voltage at the lamp may be kept constant. An adjustable diaphragm serves to regulate the amount of light which enters the lens. The parallel or slightly convergent light passes from the lens through the solution which exhibits spectroscopically characteristic absorption bands or zones. The light then passes through a suitable filter which transmits light in a region corresponding as accurately as possible to an absorption band of the material in solution. The radiant energy which has been transmitted by the solution and the spectral filter falls on the

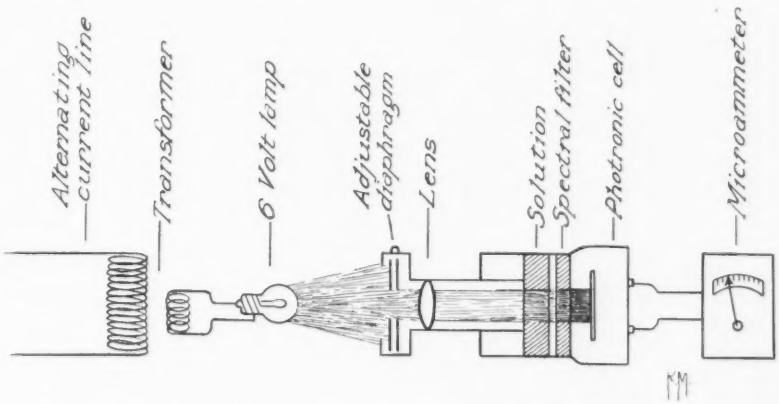

FIG. 1. DIAGRAM OF THE ESSENTIAL PHYSICAL FEATURES OF THE PHOTELOMETER

photronic cell and causes deflection of the current-measuring instrument. By varying the concentration of the material in solution the readings of the micro-ammeter may be converted into a graphic portrayal or tabulation showing the relationship between concentrations and readings on the micro-ammeter.

THE NEW IMPROVED PHOTELOMETER

The new type of photelometer differs from the photo-electrometer with one stage of amplification in two essential respects: (1) a photronic cell (Weston Electrical Instrument Company, Newark, N. J.) replaces the photo-electric cell (P. J. 24, General Electric Company or Westinghouse Electric Company) and the one stage of amplification, and (2) a specially constructed transformer,

so built as to keep the voltage (6 volts) delivered at the incandescent bulb constant within 1 or 2 per cent, and operating on the ordinary 110–115 volt alternating current circuits generally available in all communities.

Figure 2 is a view of the instrument* photographed directly from above and shows:

a. Lamp-house. This contains a 6–8 volt Mazda 50 candle power bulb and has a mechanism for adjusting the position of the lamp with reference to a double convex lens so that the light which falls on the photronic cell may be parallel or slightly convergent.

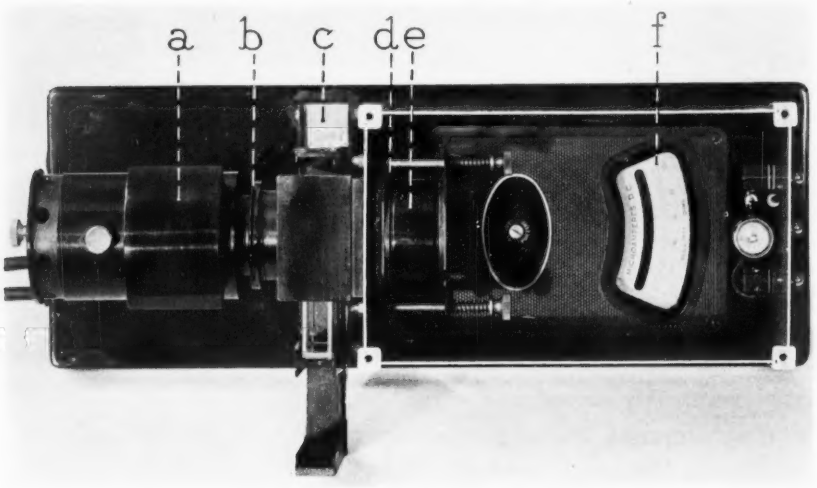

FIG. 2. THE PHOTELOMETER

a, lamphouse; *b*, iris diaphragm; *c*, carriage for holding solutions; *d*, spectral filter; *e*, photronic cell, and, *f*, micro-ammeter.

b. Diaphragm and lens. An iris diaphragm controls the amount of light which enters the lens placed immediately behind the diaphragm. The diaphragm is adjusted so that the light received by the photronic cell causes a

* Certain mechanical changes conducive to portability as well as to ease and certainty of operation have been incorporated since the time of the meeting of the Society in Milwaukee. These additional features are concerned with the proper mounting of the transformer on the base of the instrument, reduction in the number of switches, and the facility with which spectral filters or absorptive solutions may be changed and substitutes placed in proper position in front of the photronic cell.

deflection of the full scale of the current-measuring instrument (micro-ammeter) when a blank cell or container holding the solvent is placed between the source of light and the photronic cell.

c. Slide holder or carriage. This is for the reception of containers holding solutions to be measured. The movable carriage contains three compartments, one compartment of which may be used for the standard cell holding the solvent. The other two compartments are then available for rapid insertion of the solutions under examination. The containers for holding solutions are as uniformly as possible 1 cm. thick, as measured internally. Containers (spectroscopic absorption cells) which are quite uniform in this particular are obtainable commercially and at relatively small expense. It is obvious that the thickness of all solutions under test must be the same, otherwise the factor of change of thickness would have to be considered. Since the factor of thickness of solution is of importance, containers should be selected which have the same measurement of width (or thickness) when measured internally by suitable calipers. If cells of different internal thickness (D_1 and D_2) are used, correct calculations of the concentrations (C_1 and C_2) may be made in terms of the readings of the current (A_1 and A_2) on the micro-ammeter from the formula

$$\frac{C_1}{C_2} = \frac{\dfrac{-\log A_1}{D_1}}{\dfrac{-\log A_2}{D_2}} \quad \text{or} \quad \frac{C_1}{C_2} = \frac{-\log A_1}{-\log A_2} \times \frac{D_2}{D_1}$$

d. Spectral or absorptive filters. The spectral filters, whether of colored glass, Wratten filters or colored solutions, are placed directly in front of the photronic cell. The mechanical arrangement of the apparatus is such that the filter fits snugly against the photronic cell, thus preventing the admission of any stray light which might enter by reason of the incandescent lamp used to illuminate the scale of the micro-ammeter.

e. Photronic cell. The Weston photronic cell consists essentially of a thin metallic disk on which there is a film of light-sensitive material. The metal disk forms the positive terminal and a metallic collector ring in contact with the light-sensitive surface forms the negative terminal. There are no separate anode and collector plates, or evacuated or gas-filled space, as in the ordinary type of photo-electric cell. The action of light impinging on the sensitive cell appears to be entirely electronic. Tests made indicate that no chemical or physical change takes place, and therefore the life of the photronic cell seems to be unlimited. The current output of the cell is linear for values of the external resistance which are small (of the order of 10 per cent or less) when compared with the resistance of the cell. These cells, in the original form of manufacture, produce on the average about 1.4 micro-amperes per foot-candle of illumination uniformly distributed over the sensitive surface when connected to a relatively low external resistance. The latest type of cell contains no central nonsensitive area and is considerably more sensitive than the original form.

f. Micro-ammeter. The two wires leading from the photronic cell are connected to the positive and negative terminals respectively of the micro-ammeter (Weston Electric Instrument Co., Type No. 440), the scale of the instrument being divided into 100 parts. In order that the needle may be sufficiently damped to permit of rapid readings, a suitable external resistance (critical damping resistance) is properly placed in the circuit containing the micro-ammeter.

g. Lamp for illumination of the scale and magnifying lens. A 6–8 volt, 3 candle power bulb is used for the illumination of the scale. A lens, of low magnifying power and cut to an appropriate shape, is inserted in the cover of the instrument (fig. 2).

THE USE OF THE PHOTELOMETER IN THE DETERMINATION OF THE AMOUNT OF HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD

In order that photelometry may be carried out with accuracy in the determination of any substance three things are essential: (1) the solution must possess at least one absorption band or zone; (2) a spectral filter (Wratten, for example) or solution must be available which transmits light only in the region of one absorption zone of the material in solution which is under test, and (3) a method of obtaining a definite, known concentration of material in solution must be at hand to serve as a standard; this standard, therefore, by subsequent known degrees of dilution, provides a means of altering the concentrations in definite amounts.

Preparation of the sample of blood. Twenty cubic centimeters of the diluting fluid (0.1 per cent solution of sodium carbonate in water) are measured accurately into a suitable container such as a 50 c.c. centrifuge tube or Erlenmeyer flask. Preferably the blood should be taken from a vein. It is therefore most convenient to make the dilutions at the same time that the patient is being bled for tests on the serologic or chemical changes of the blood. The blood may be pipetted from a small tube immediately after it has been placed in it. Any long pipette with a capillary bore that is accurately marked for 0.1 c.c. may be used. Exactly 0.1 c.c. of blood is to be delivered by the pipette into the 20 c.c. of carbonate solution. It is obvious that this procedure must be done accurately and quickly. The tube is then shaken to facilitate complete dilution. The hemoglobin is converted immediately into oxyhemoglobin. Further details may be obtained from the paper by Sanford and Sheard.[5]

Spectrophotometric curve of oxyhemoglobin. Curves 2 and 3 of figure 3 show the characteristic absorption bands in the visible spectrum of oxyhemoglobin.

The curves give data for two concentrations of hemoglobin in water. The absorption bands have minimal transmission values at 578 and 542 millimicrons respectively.

Spectral filter. Curve 4 (fig. 3) shows the spectral transmission of Wratten filter number 74, and, furthermore, shows that it transmits light only in the region of oxyhemoglobin which has its maximal absorption at 542 millimicrons.

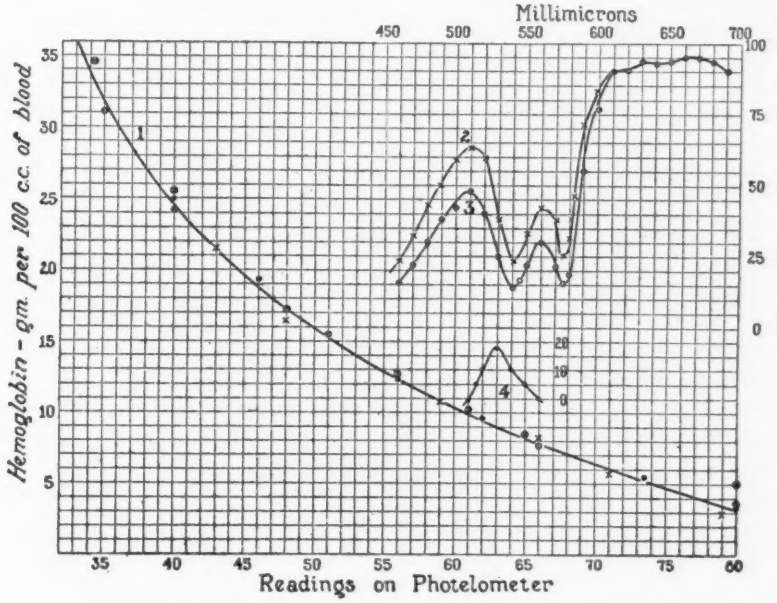

FIG. 3. DETERMINATION OF HEMOGLOBIN

Curve 1, relationship between the readings on the photelometer and the grams of hemoglobin determined by the method of Van Slyke. Curves 2 and 3, spectrophotometric transmissions of diluted blood. Curve 4, spectrophotometric transmission of spectral filter used in the quantitative estimation of hemoglobin.

Standardization of hemoglobin. The oxygen capacity method of Van Slyke gives, by suitable calculation and with a probable error of about 1 to 2 per cent, the grams of hemoglobin for each 100 c.c. of blood. The reader is referred to the papers of Van Slyke[4, 8] on gasometric determinations.

Correlation of grams of hemoglobin per 100 c.c. of blood and the readings on the photelometer. Curve 1 of figure 3 gives data showing the values of the readings on the photelometer in terms of the grams of hemoglobin per 100 c.c. of blood. Accurate determinations of the grams of hemoglobin were made of the blood

of several individuals by the Van Slyke method. Various but accurately measured amounts of blood were taken from the samples obtained at the time of the gasometric (Van Slyke) determinations and were diluted with 0.1 per cent sodium carbonate to 20 c.c. in each case. These dilutions of blood were then placed in the containers (1 cm. thick) which, in turn, were introduced into the sliding carriage of the photelometer after the instrument had been adjusted to read a full-scale deflection (100 divisions) when a container holding the solvent only was inserted between the source of light and the photronic cell. The accuracy of the data of curve 1, figure 3, is dependent obviously on the accuracy of the determinations of content of hemoglobin by the method of Van Slyke, and on the skill and care with which the dilutions of samples of blood are made for use in the photelometer.

The method of making a reading. The spectroscopic absorption cell is cleaned thoroughly with water, alcohol and ether. The container filled with water is placed in the middle compartment of the sliding carriage of the photelometer and the iris diaphragm adjusted until the pointer of the micro-ammeter is exactly at the 100 mark. Containers filled with diluted blood are then inserted in the right and left compartments of the carriage and the carriage is moved and the readings of the micro-ammeter are made. The accuracy of setting of the micro-ammeter at the 100 division mark can be checked at any time if the container holding the solvent (water in this case) is allowed to remain in the middle compartment of the carriage The reading on the micro-ammeter obtained by the insertion of diluted blood is translated readily into grams of hemoglobin in each 100 c.c. by referring either to the curve (fig. 3) or to a tabulation which can be made from the data of the curve.

In a series of experiments made to determine any fading effects due to standing of solutions of blood, it was found that there was less than 2 per cent change in the values of the grams of hemoglobin present after two or three hours' interval. Apparently any fading which occurs takes place during the first two or three hours, since readings taken four to eight hours later show no further changes. However, it is excellent practice, as well as a necessary procedure in measurement of certain types of solution, to make the readings as soon as possible after the dilutions are made.

THE USE OF THE PHOTELOMETER IN THE DETERMINATION OF BLOOD SUGAR IN MILLIGRAMS PER 100 C.C. OF BLOOD

As examples of the quantitation of solutions of materials, other than hemoglobin, which possess color and therefore one or

more absorption bands or zones, we present the results obtained with the photelometer on the determination of dextrose in blood and of creatinine in blood. The blue solution for the determination of dextrose is obtained by the method (given in detail in succeeding paragraphs) of Folin and Wu.[3]

Principle. The protein-free blood filtrate is heated in a solution of alkaline copper, using a special tube to prevent reoxidation. The cuprous oxide formed is treated with a solution of phosphomolybdic acid. A blue colored solution is obtained.

Solutions. Solution 1, stock solution: Dissolve 2.5 gm. of benzoic acid in 1 liter of boiling water and cool. Transfer to a bottle; the solution will keep indefinitely. Dissolve 1 gm. of pure glucose in about 50 c.c. of the benzoic acid solution. Transfer to a 100 c.c. volumetric flask, rinse, and fill to the mark with the benzoic acid solution. This is the standard (1 per cent) stock solution. Label and preserve. This solution seems to keep indefinitely. Transfer 3 c.c. of the stock solution, by means of an Ostwald pipette, to a 200 c.c. volumetric flask; fill to the mark with saturated benzoic acid solution and mix. The diluted solution so obtained, which contains 0.15 mg. of glucose per cubic centimeter, is a suitable standard for most determinations of blood sugar. Use 2 c.c. for each determination.

Solution 2: Alkaline copper solution. Dissolve 40 gm. of anhydrous sodium carbonate in about 400 c.c. of water, and transfer to a liter flask. Add 7.5 gm. of tartaric acid, and when the latter has dissolved, add 4.5 gm. of crystallized copper sulphate; mix and make up to a volume of 1 liter. If the carbonate is impure, a sediment may be formed in the course of a week or so; in this event decant the clear solution into another bottle.

Solution 3: Special phosphomolybdic acid solution. Transfer to a liter beaker 35 gm. of molybdic acid and 5 gm. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for twenty to forty minutes so as to remove almost all of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c., and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.

Procedure for the preparation of the solutions for the quantitation of blood sugar. Transfer 2 c.c. of the tungstic acid blood filtrate to a blood sugar test tube, and to another similar test tube (graduated at 25 c.c.) add 2 c.c. of standard sugar solution containing 0.15 mg. of dextrose. To each tube add 2 c.c. of the alkaline copper solution. The surface of the mixtures must reach the constricted part of the tube. Transfer the tubes to a boiling water bath and heat for 6 to 8 minutes. Remove the sugar tubes and add immediately (that is, before cooling) 2 c.c. of the molybdate phosphate solution. When the cuprous oxide is dissolved, cool, and dilute the resulting blue solutions to the 25 c.c. mark, insert a rubber stopper, and mix. It is essential that adequate attention be given

to this mixing because the greater part of the blue color is formed in the bulb of the tube.

Quantitation of blood sugar by the photelometer. Curves 2 and 3 of figure 4 show the values of the spectrophotometric transmissions, at wavelengths from 700 to 440 millimicrons, of two different concentrations of preparations for the determination of blood sugar. Both curves exhibit a definite absorption zone

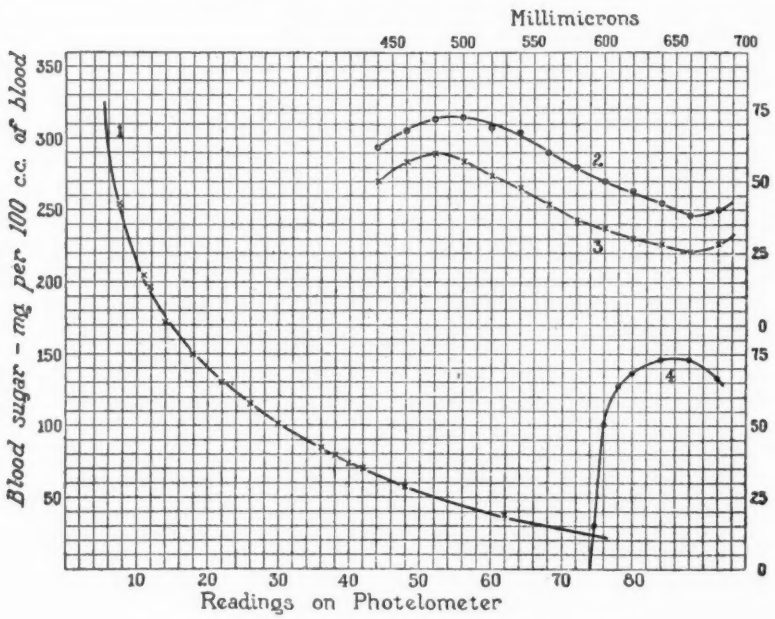

FIG. 4. DETERMINATION OF BLOOD SUGAR

Curve 1, relationship between concentrations of blood sugar and the readings on the photelometer. Curves 2 and 3, spectrophotometric transmissions of blood sugar solutions prepared by the method of Folin and Wu. Curve 4, spectrophotometric transmission of the spectral filter used in the quantitation of blood sugar.

with a minimal transmission at approximately 660 millimicrons. Curve 4 shows the spectral transmission of Wratten filter number 29 and demonstrates that the filter transmits light in maximal quantity in the region 660 to 660 millimicrons, or in the region in which the preparations of blood sugar are markedly absorptive. Curve 1, figure 4, was obtained by plotting the values of blood sugars, as determined colorimetrically, as ordinates and the corresponding readings of the photelometer as abscissae. Or, for purposes of calibration, definitely determined dilutions of samples of the standard solution may be made

and a curve plotted showing the relationship between milligrams of blood sugar per 100 c.c. of blood and readings on the photelometer. Because of fading or loss of color in solutions prepared for the determination of blood sugar, it is essential that a definite time schedule be adopted for the accurate determination of a curve similar to curve 1 of figure 4. For example, a ten-minute interval between preparation and reading on the photelometer may be selected. Irrespective of the regimen adopted, strict adherence to the schedule must be maintained in either colorimetric or photelometric estimations of solutions which change in color value on standing.

THE USE OF THE PHOTELOMETER IN THE DETERMINATION OF CREATININE IN MILLIGRAMS PER 100 C.C. OF BLOOD

The yellow solution of creatinine, obtained by treatment of protein-free filtrate with alkaline picrate (by the methods given here in some detail) may be quantitated by the photelometer.

Principle. By colorimetric methods, a portion of the blood filtrate is treated with alkaline picrate solution and the color developed compared with that of a standard. In making determinations of creatinine with the photelometer it is necessary to determine a curve showing the relationship between milligrams of creatinine per 100 c.c. of blood and the readings on the micro-ammeter.

Solutions. Solution 1: Stock standard creatinine solution is obtained by dissolving 1.61 gm. of creatinine zinc chloride in 1 liter of N/10 HCl.

Solution 2: Dilute standard creatinine solution. Transfer to a liter flask 6 c.c. of the stock standard creatinine solution, add 10 c.c. of normal hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add 4 or 5 drops of toluene or xylene; 5 c.c. of this standard contains 0.03 mg. of creatinine.

Solution 3: Saturated picric acid solution is obtained by dissolving 1.50 gm. of purified picric acid in 100 c.c. of water.

Procedure for the preparation of solutions for the quantitation of creatinine. Transfer 25 (or 50) c.c. of a saturated solution of purified picric acid to a small, clean flask, add 5 (or 10) c.c. of 10 per cent sodium hydroxide and mix. Transfer 10 c.c. of blood filtrate to a small flask, or to a test tube, and dilute the standard to 20 c.c. Then add 5 c.c. of the freshly prepared alkaline picrate solution to the blood filtrate and 10 c.c. to the diluted creatinine solution. Let stand for eight or ten minutes and make the determinations with the photelometer. The photelometric determinations should be completed within a specified time (not over fifteen minutes) from the time the alkaline picrate was added. Therefore it is not advisable to work with more than three to five blood filtrates at a time.

In the case of unusual bloods representing retention of creatinine, take 10 c.c. of the standard plus 10 c.c. of water, which covers the range of 2 to 4 mg.

of creatinine per 100 c.c. of blood; or 15 c.c. of the standard plus 5 c.c. of water by which 4 to 6 mg. can be estimated. By taking the full 20 c.c. volume from the standard solution at least 8 mg. can be estimated.

Quantitation of creatinine by the photelometer. Curves 2 and 3 of figure 5 show the values of the spectrophotometric transmissions in the visible spectrum of two different concentrations of preparations for the determination of creatinine. Both curves show a definite absorption zone in the short wave length region of the spectrum. Curve 4 shows the spectral transmission of Wratten

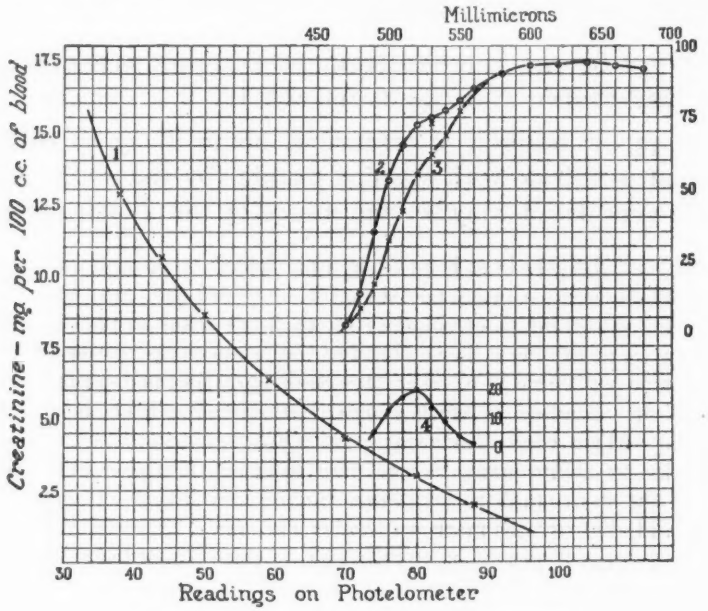

FIG. 5. DETERMINATION OF CREATININE

Curve 1, relationship between concentrations of creatinine and readings on the photelometer. Curves 2 and 3, spectrophotometric transmissions of two solutions of creatinine. Curve 4, spectrophotometric transmission of spectral filter used in the quantitative estimation of creatinine.

filter number 63 and demonstrates that the filter transmits light in maximal quantity in the region 500 to 530 millimicrons or in a portion of the region in which the preparations of creatinine are markedly absorptive. Curve 1 was obtained by plotting the values of creatinine, as determined by dilution of a preparation containing 12.8 mg. of creatinine by colorimetric methods, against the readings on the photelometer. The original creatinine preparation was diluted with amounts of tungstic acid to give values of creatinine corresponding to 10.6, 8.5, 6.4, 4.3 and 2.1 mg. per 100 c.c. of blood respectively.

Because of fading or loss of color in preparations of creatinine, it is essential to adopt a definite time schedule.

SUMMARY

Information and data have been presented concerning the following:

Fundamental principles of photo-electric photometry, with a résumé of their application, as previously made by us, to the development of the photo-electric hemoglobinometer and the photo-electrometer with one stage of amplification.

The development of a new type of instrument, the photelometer, using as a source of illumination a 6 to 8 volt, 50 candle power Mazda lamp operated at constant voltage by means of a specially designed transformer and a photovoltaic cell (photronic cell), in direct combination with a micro-ammeter, as the optico-electrical system for measuring the amount of light transmitted by various substances in solution.

Details concerning the construction and operation of the photelometer, and such accessories as are essential or desirable for ease of operation and accuracy of results.

The advantages of the use of the photelometer and appropriate spectral filters in clinical and chemical laboratories, as illustrated by data and curves relative to the quantitative estimation of hemoglobin, blood sugar and creatinine.

The applicability of the principles of photo-electric photometry, as exemplified in the photelometer, to the quantitative determinations of various substances in solution which possess at least one characteristic absorption band or zone in the visible spectrum and also to the quantitative estimation of materials in suspension.

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FLOCCULATION REACTIONS FOR SYPHILIS*

Comparative Results with Six Flocculation and Two Complement Fixation Tests

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Innumerable series of tests have been made on blood specimens since the first complement fixation reaction for syphilis was reported by Wassermann in 1906. The almost simultaneous development of the flocculation principle by Michaelis for the diagnosis of syphilis started the controversy between the proponents of the two types, and this still persists. Refinements in the technic of these two types of tests have resulted in perhaps the most accurate diagnostic laboratory procedures known for any one condition.

The constant urge that these tests be made more sensitive and more simple has developed the large number of modifications of both the complement fixation and flocculation or precipitation tests, with only a few attempts toward standardization.

Realization that both types of tests are physico-chemical in nature, and in principle are quite if not entirely similar may result in ultimate standardization to one or two tests of each type. The appreciation that the same variables are largely present in the different tests, as stated by Eagle,[2] and that experience by trial and error should fix these variables within narrow limits may result in a closer approach to the ideal test as outlined by Kilduffe.[6]

Many of the published reports evaluate other tests in terms of

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a favorite procedure. This is naturally true of reports by the originators of the different tests, and by their expert assistants, as both have developed a surpassing technic in the procedure in which they are particularly interested, as compared with other tests.

Perhaps every clinical laboratory with any considerable volume of work has run a series of blood specimens with different tests to determine the procedures most satisfactory for them. That idea has been carried out for several years in this laboratory, and has crystallized in the present report.

The present series of blood tests was carried out to determine the relative value of the different procedures in the hands of well trained, competent but not expert technicians.* Similar conditions of personnel undoubtedly prevail in many other qualified laboratories, also the majority of the reactions under discussion are reported by such laboratories. The different tests reported were carried out previously for varying lengths of time until dependable results had been obtained.

One thousand specimens of blood were run by the six flocculation or precipitation tests and the two complement fixation tests, as described below. All tests were done on the same specimen of blood, and for this purpose ten cubic centimeters of venous blood were obtained from a routine series of patients in the clinics and wards of the University Hospitals.

The WaR test is a complement fixation procedure which has been carried out routinely for almost twenty years in this laboratory, with but minor alterations. Two antigens, one cholesterolized, water bath incubation, and the sheep's cell system is employed. The Kolmer[8] quantitative test has been carried out for several years with close adherence to the original technic.

The Eagle,[1] Hinton,[3,4] Kahn standard,[5] Kline,[7] Meinicke (M.K.R.),[10] and Müller (M.B.R. II)[11] tests have been carried out according to the described technic. Antigens† for the Eagle,

* An expert technician is considered in this discussion to be exceptionally well trained for a particular procedure, and who devotes the major part or all of his time to that procedure.

† Drs. Hinton, Kahn and Kline kindly furnished us with samples of their respective antigens which served as checks for the antigens prepared by us.

Hinton, Kahn, Kline, Kolmer and WaR tests as used in this series were prepared in this laboratory. The Meinicke and Müller antigens were purchased in the open market, as no adequate directions could be found for their preparation. The Kline antigen was used for both the diagnostic and exclusion tests.

Among the thousand cases were a number of individuals with a luetic history or positive physical findings for primary lesions, secondary eruptions, and various tertiary and congenital manifestations. No effort, however, has been made to classify the cases into groups other than to prove or disprove the presence of syphilis in every way possible.

TABLE 1

RESULTS OF THE VARIOUS TESTS ON 1,000 BLOOD SPECIMENS

	ALL REACTIONS AGREE		ONE OR MORE REACTIONS DISAGREE		
History—luetic	Positive	Negative	Positive	Positive	Negative
Reactions	Positive	Negative	Negative		
Number of bloods	224	564	11	158	43
Percent	22.4	56.4	1.1	15.8	4.3
Total percent	79.9			20.1	

Table 1 enumerates the cases in which there was complete serological agreement, positive and negative, with the history; also complete disagreement. No distinction was made for the degree of positive reactions, as emphasized by Kolmer.[9] Eleven blood specimens were from cases with a definite luetic history, and with treatment apparently sufficient to produce negative reactions. Partial serological agreement and the false positive reactions are considered separately. Those serological tests which gave positive reactions in which no possible evidence could be elicited of syphilis were considered as probably false.

In table 2 those cases with a positive luetic history but with disagreement in reactions are analysed. Many of these cases had had more or less treatment, and were largely tertiary or

latent in clinical manifestations. Two cases with primary lesions and a positive darkfield examination for *Treponema pallidum* were in this group, with the syphilitic reagin apparently just beginning to develop. The balance of the group shows that a large majority gave positive reactions of varying degree with five or more tests. The relative order of sensitivity is interesting, as it shows that in this group the flocculation tests gave definitely the larger number of positive reactions. It is in these types of cases that the tests have their greatest value, to deter-

TABLE 2

REACTIONS OF 158 BLOODS WHICH GAVE VARYING RESULTS WITH DIFFERENT TESTS

Luetic history, positive

TEST	POSITIVE	PERCENT OF 1,000 BLOODS	ORDER OF SENSITIVITY
WaR	20	2.0	8
Kolmer (quantitative)	86	8.6	7
Eagle	116	11.6	4
Hinton	118	11.8	3
Kahn	98	9.8	6
Kline	135	13.5	1
Meinicke	129	12.9	2
Müller	111	11.1	5

Total positive reactions for this group: 813
Positive reactions per blood: 5+

mine diagnosis and to control treatment. But in this group the greatest difficulties arise.

The degree of sensitivity which apparently can be more or less controlled has been another point of controversy in these tests of non-specific nature. The ideal test will give no false positive reactions. Forty-three blood specimens from cases with non-luetic histories gave fifty-nine positive reactions of varying degrees with the different tests, as shown in table 3. The Kline and Müller tests gave very definitely more positive reactions in these cases. This is not parallel, however, to the order of sensitivity as shown in table 2. This difficulty of the hypersensitive

antigen and the false positive reaction is considered by Kilduffe[6] as a problem for the clinician, who can best evaluate the laboratory results. Error in technic may account for a certain number but not all of the erroneous results obtained.

TABLE 3

ONE OR MORE REACTIONS POSITIVE

Luetic history, negative

TEST	NUMBER OF FALSE POSITIVE REACTIONS
WaR	1
Kolmer quantitative	1
Eagle	1
Hinton	1
Kahn	3
Kline	19
Meinicke	3
Müller	29
Total	58

Number bloods in this group: 43
Positive reactions per blood: 1+

COMMENTS

The impressions gained from previous work and from this series of tests are that the Kolmer quantitative test is the most valuable complement fixation procedure for the diagnosis of syphilis we have used. The technic is, however, quite difficult, and this unfortunately must prevent its more general use.

Among the flocculation tests the Eagle technic stands out for its relative simplicity, specificity, and ease of reading. The Müller reaction also is easily read, but has given us a much greater number of non-specific reactions. The Meinicke test probably ranks next to the Eagle test, but the fact that this antigen and also the Müller antigen must be purchased without any knowledge of their age or care since they were prepared and with an apparent difference encountered in different lots all act to the disadvantage of both.

The Hinton and Kahn tests show a high degree of specificity, but both were found to be rather difficult to read. There is but little to choose between these two tests except perhaps the greater simplicity of the Hinton test with only one tube.

The use of the paraffin rings in the Kline test set this procedure apart from the others and while we found it an easy technic to carry out there was a definite tendency to give non-specific reactions. However in luetic cases it ranked first in sensitivity.

CONCLUSIONS

This series has shown the advantage of performing a complement fixation test and a flocculation test on every blood specimen as supplemental to each other.

The flocculation tests have a definitely higher degree of sensitivity than the complement fixation tests in treated and latent cases of syphilis.

The majority of the tests concerned have a high degree of specificity.

All eight tests require a high degree of technical skill, and increasing experience with any test enhances its value.

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A SURVEY OF 1000 GONOCOCCUS COMPLEMENT-FIXATION TESTS PERFORMED WITH THE SERUMS OF MALE PATIENTS IN AN OUTPATIENT CLINIC*

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The object of this study was to analyze the results of routine complement-fixation tests performed with the blood serums of male patients in a large clinic treating ambulatory cases only, and to establish, if possible, with the aid of a detailed clinical study, the practical value of this laboratory procedure. The analysis is based upon a study of 1000 tests performed with the serums of as many patients.

The gonococcus antigen used was a commercial preparation which consisted of a suspension of pure cultures of gonococci. It was prepared from a number of strains of the organism isolated from acute and chronic cases. The various strains incorporated in this antigen were so selected as to insure the applicability of the antigen in any cases of gonococcal infection. The procedure employed is outlined in table 1.

The tubes were placed in the ice-box over night, and the results were read the following morning. Depending upon the degree of the hemolytic inhibition, the results were recorded in accordance with the generally adopted scheme for complement-fixation, namely: four-plus, three-plus, two-plus, one-plus, trace or doubtful, and negative.

All the serums upon which this study is based were tested by the Kolmer complement-fixation and Kahn antigen precipitation tests in addition to the gonococcus complement-fixation.

The cases studied were grouped for the purpose of analysis in accordance with the results of the serologic tests.

NEGATIVE BY ALL THE TESTS USED

Serum specimens from six hundred patients, or 60 per cent of the series studied gave negative results by all the tests employed.

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Three hundred histories taken at random were examined. It was found that in seventy cases, or 23 per cent of the 300, the diagnosis of acute gonococcal urethritis was made; three, or 1 per cent, gonococcal prostatitis; three, or 1 per cent, gonococcal urethritis, chronic. Of the seventy cases of acute urethritis six, or 2 per cent of the 300, were diagnosed as posterior urethritis. Sixty patients, or 20 per cent of the 300, denied having had a gonococcal infection, while eighty-one, or 27 per cent of the 300, admitted one or more attacks. The period elapsing since the last attack of the gonorrheal infection among the patients who admitted having had the infection varied from one to twenty-

TABLE 1

REAGENTS	1ST TUBE	2ND TUBE	CONTROL TUBE
	cc.	*cc.*	*cc.*
Patient's serum 1:5 dilution	0.5	0.25	0.5
Antigen 1:20 dilution	0.15	0.15	None
Saline	0.35	0.60	0.85
Complement 1:50 dilution	1.0	1.0	1.0
Incubate for one hour in a water-bath at 37.5°C. then add			
Sheep cells, 5 per cent suspension	0.5	0.5	0.5
Amboceptor 1 dose, 1:1000 dilution	0.5	0.5	0.5
Incubate for one hour in a water-bath at 37.5°C.			

five years, and the number of attacks varied from one to six. All had received treatment, which, according to the statements given, resulted in complications of the usual type in a noteworthy percentage of instances. The examination of the patients whose condition was diagnosed as gonococcal infection was made from two days to four months following the date of the alleged exposure, the greater percentage varying from one to four weeks. Nothing directly or indirectly connected with the gonorrheal infection could be discerned from the histories of the remaining 50 per cent of the cases of this group.

The most pertinent points obtained in the above analysis are recapitulated in table 2.

The above analysis indicates that complement-fixation by the antigen consisting of a suspension of unwashed gonococci fails to yield positive results in 25 per cent of cases with acute gonococcal urethritis even as late as four weeks following the infection. Most of the cases had an anterior infection, though some posterior infections of the same duration likewise failed to react positively with the complement-fixation procedure as used in this study. Since nearly all the cases with a history of the gonococcal infection as far back as one to twenty-five years, with and without ensuing complications (but free from active infection), do not respond positively to the gonococcus complement-fixation, it

TABLE 2

	per cent
Negative gonorrheal complement fixation results were obtained in:	
Gonococcal urethritis, acute, anterior	21
Gonococcal urethritis, acute, posterior	2
Gonococcal urethritis, chronic	1
Gonococcal prostatitis, chronic	1
Total per cent of cases with active gonorrheal infection	25
Cases free from active gonorrheal infection, but with history of past infection	25

appears reasonable to assume that the persistence of the gonococcus antibody is of a limited duration.

CASES POSITIVE BY THE GONOCOCCUS COMPLEMENT-FIXATION ONLY

Cases with strongly positive reactions. (Four plus—four plus to three plus—two plus, in the first and second tubes respectively)

Serums of ninety patients studied, or 9 per cent of the entire series, gave reactions of the intensities indicated above. In examining the histories of the patients whose serums are included in this group, the following important information was considered: (1) History of previous gonococcal infection, (2) date of exposure, (3) urethral discharge, (4) results of the two glass urine test, (5) microscopic urine examination, mainly for the presence of pus,

(6) glandular involvement, (7) examination of the prostatic discharge, and (8) examination of the urethral discharge by the methylene blue method.

The most important information gathered from the clinical histories of the ninety cases of this group can be conveniently presented as in table 3.

Forty-two of the ninety patients admitted having had previous gonococcal infections with periods of time varying from four months to fifteen years. No account was taken of the number of previous attacks of gonorrhea. The examination of the patients of this group was made from two days to six weeks following the

TABLE 3

DIAGNOSIS	NUMBER OF CASES
Acute gonorrheal urethritis	41
Acute gonorrheal urethritis and syphilis, treated, seronegative	5
Chronic prostatitis	16
Gonorrheal urethritis, posterior	11
Gonorrheal urethritis, prostatitis, and epididymitis	3
Gonorrheal urethritis, prostatitis, and epididymitis, and syphilis, treated, seronegative	3
Urethral gland infected	1
Left town (were under observation for gonorrheal infection)	3
Enlarged prostate (under observation for gonorrheal infection	4
Non-venereal	3

alleged exposure. The strong gonorrheal complement-fixation reactions with the serums of the last three cases shown in table 3 indicate the possibility of either a nondivulged history of a comparatively recent attack which may have been cured or suppressed shortly before the patients reported at the Institute, or of the reactions being non-specific. It is probable that upon successive repeated tests the reactions might be found to disappear spontaneously, as is the case with many of the non-specific fixation reactions obtained with the beef-heart antigen. This phase of the problem deserves a thorough study.

Generally, however, the analysis of the results of the serum-reactions included in this group indicates that strong and moder-

ate gonococcus complement-fixation reactions are predominately specific and indicate the existence of an active or recently active gonorrheal infection. This leads us to believe that cases suspected of having a gonococcal infection but lacking in clinical and bacteriological evidence, yet yielding strongly positive gonococcus complement-fixation reactions, should be investigated thoroughly for foci of gonococcal infection. If thorough repeated examinations fail to disclose any evidence of a gonococcal infection, the complement-fixation test should be made several times. If the positive reaction disappears spontaneously, the original positive reaction may be disregarded. If it persists, a further painstaking study of the case for gonococcal activity remains warranted.

Cases with serum reactions of weak intensity. (Two plus—two plus to one plus—one plus, in the first and second tubes respectively)

Serums of forty of the patients studied, or 4 per cent of the series, gave gonococcus complement-fixation reactions of weak intensity. A study of the clinical histories of these patients showed that while the larger percentage had a diagnosis bearing upon the gonorrheal infection (mostly acute urethritis), non-specific reactions began to make their appearance to a noteworthy extent. Thus, six cases were diagnosed as no disease found, though three of these patients admitted having had gonorrheal infections five years prior; three cases were diagnosed as non-syphilitic penile lesions, and were free from clinical evidence of urethral involvement; one non-syphilitic, alcoholic pellagra; two, syphilis, suspected, not proved, no urologic involvement clinically.

Twelve reactions, or 30 per cent of this subgroup, accordingly, must be referred to as non-specific. Upon repeating tests some of these weakly positive reactions might prove to be transient. However, from the view-point of routine study, the impression was gained that complete reliance upon the specificity of the gonococcal complement-fixation reaction must be limited to tests of the strong and moderately strong intensity of fixation.

Cases with doubtful serum reactions. (One plus—trace; one plus—negative; trace—trace; trace—negative; in the first and second tubes respectively)

Serums of fifty of the patients studied yielded gonococcus complement-fixation reactions which are usually designated as doubtful. The diagnoses of these cases are tabulated in table 4.

It is seen from table 4 that thirty-two cases, or 64 per cent of this group, were entirely free from active gonococcal infection. This is more than double the percentage of non-specific reactions found in the group just preceding. The percentage of non-specific reactions of doubtful and weak intensity might be found to be considerably greater among patients of polyclinics. In routine gonococcus complement-fixation, therefore, weakly positive reactions should be reported as doubtful, unless the reactions persist, and doubtful results should be reported as negative. The clinician must bear in mind, however, that negative gonococcus complement-fixation in patients suspected of having an active gonococcal focus are of no more diagnostic value than negative routine serologic results are in the cases of suspected syphilitic patients. It is regretable, yet true, that in both instances the laboratory still fell short of its aim, and the burden of decision fell entirely upon the clinicians.

TABLE 4

DIAGNOSIS	NUMBER OF CASES
Gonorrheal urethritis, chronic	5
Gonorrheal urethritis, acute	7
Gonorrheal urethritis, posterior	2
Stricture of the urethra	2
Prostatitis, chronic	2
Non-venereal	32

SUMMARY AND DISCUSSION

In evaluating the study presented in this paper, we are lead to conclude that in routine clinical work gonococcus complement-fixation results are biologically specific only when the intensity of

the reactions is strong or moderately strong. Weak and doubtful reactions are non-specific to a very high degree. Furthermore, in some cases of syphilis of long standing even strongly positive gonococcus complement-fixation reactions appear. This is in disagreement with the opinion of Kolmer[3] who believes that in gonococcus complement-fixation "positive reactions including the weakly positive ones are invariably true and specific." Barringer, Strauss and Crowley[1] think that in gonococcus complement-fixation "positive complement-fixation test readings, especially three plus and four plus and over, have definite clinical value which can be relied on." They state further that even a two plus reaction in the later subacute and chronic stages generally means "a full blown, typical gonorrheal invasion which is running its usual course". . . . and "as the clinical picture subsides and the bacteriologic findings become negative, the complement-fixation drops to a one plus reading, then to plus or minus, to minus trace, to minus." Accordingly, the authors evaluate a plus or minus reading as a very weakly positive finding.

Such a method of evaluating the results of gonococcal complement-fixation, when a mixed suspension of the organisms is used, is most probably justified in a follow up study of known cases of gonorrhea that are hospitalized. However, when using the same complement-fixation procedure as a routine on the admission of cases in an outpatient department for the purpose of "picking up" those patients who show no clinical evidence of a gonococcal infection, the evaluation of the laboratory results reported by Barringer and her collaborators will, in our opinion, prove hazardous.

A large percentage of cases with acute gonococcal infection failed to yield positive fixation reactions. A few instances of posterior urethritis likewise gave negative complement-fixation reactions with the unwashed gonococcal antigen. It is evident, therefore, that in the early stages of the gonococcal infection negative complement-fixation results are of no value. This seems to be true equally of cases with the first or repeated infections. On the other hand, persistent strong or moderately strong complement-fixation reactions may be safely regarded as indicative of

residual active infection, or of latent foci of the epididymis, prostate, vesicles, etc. Opposed to this is the wide spread view that about 25 per cent of cases clinically free from any manifestation of the gonococcal disease, regarded as cured, yield positive reactions for varying periods of time and some times indefinitely. The analysis presented in this paper fails to substantiate such an opinion.

Our belief that persistent strongly positive or moderately positive reactions are indicative of residual gonococcal activity or of latent foci is supported by the opinion of Pelouze,[5] who states that

> in repeated infections by the same *strain* there results either an antibody balance or a local toxin desensitization which makes possible for the germ to remain viable for long periods without the other evidence of infection.

Our opinion is further supported by Keyes[2] who states that gonococcus complement-fixation tests "usually remain positive two to six weeks after all clinical evidence of infection has disappeared." He dismisses the idea of the persistence of any complement-fixation reaction for gonococcal antibody which may be induced by specific vaccine treatment. Keyes states further that a more persistent positive reaction in authentically cured cases is "extremely unusual." So far as the effect of specific vaccine therapy upon the gonococcus fixation is concerned, it need not be considered in our study, since none of the patients whose serums were used received such therapy.

Cases of syphilis previously treated to negative serology show no cross-fixation with the beef heart antigen following the patient's infection with gonococci. Nor is there any evidence appearing in our study indicating extensive cross-fixation of new syphilitic infections with the gonococcal antigen. There is, therefore, truth in the contention of Neuberg[5] who thinks it is doubtful whether the positive Wassermann reaction has any effect on the outcome of the gonorrhea complement-fixation reaction. Since, however, in 4 to 5 per cent of the total cases studied by us gonococcal complement-fixation occurred with the serums of patients having positive tests for syphilis, but no clinical evidence of the gonococcal activity, the statement of Neuberg must be qualified.

In the light of our experience, the qualifying statement should be as follows: the positive reactions for syphilis appear to have no effect upon the outcome of the gonococcal complement-fixation tests, except in some instances of sero-positive syphilis of long standing.

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A MICRO METHOD FOR THE QUANTITATIVE ESTIMATION OF THE PROTEINS OF BLOOD PLASMA*

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This method for fractionation of proteins in blood plasma was developed for use with small laboratory animals when repeated estimations were desired Howe's[2] micro method requires 3 cc. of plasma or serum for complete fractionation. For the refractometric method of Robertson[3] 0.5 cc. of serum is needed; this method allows only the determination of albumin and globulin. Wu's[5] colorimetric method, based on a determination of the tyrosine content of the various proteins, requires 2 cc. and estimates only fibrin, albumin and globulin. Theorell and Widström[4] have recently published a micro method which reduces the total amount of plasma used to a little over 1 cc., but also is open to the objection that only fibrin, albumin and globulin are determined. The method offered here is based on Berglund's[1] modification of Howe's method and requires 0.7 cc. of plasma for complete fractionation or 0.4 cc. for the determination of fibrinogen, globulin and albumin.

MICRO METHOD

1.5 cc. of blood from the ear of a rabbit is allowed to drip directly into a centrifuge tube containing powdered potassium oxalate, with which it is thoroughly mixed. The tube is centrifuged at 3500 revolutions a minute for four minutes. The plasma is syphoned off through a fine glass syphon into a small straight sided vial containing a 2-holed rubber stopper through one hole of which passes the syphon, and through the other, a short glass tube connected by rubber tubing to a glass mouth piece. By this arrangement the stream of plasma through the syphon can be regulated and the plasma removed from the

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mass of cells to almost the last drop. The plasma is then distributed to previously prepared 10 cc. volumetric flasks by means of a Folin tube for micro determination of blood sugar, accurately graduated in 0.1 and 0.2 cc. graduations.

Preparation of flasks: The following amounts of anhydrous Na_2SO_4 are weighed on an analytical balance into a series of 10 cc. flasks: (1) none (2) 1.07 gm. (3) 1.42 gm. (4) 1.78 gm. (5) 2.204 gm. Into nos. 2, 3, 4 and 5 are added 0.4 cc. of a buffer solution to be described below. To all five is added water at 35° to just below the 10 cc. mark. The flasks are stored in an incubator at 35° in order to maintain the Na_2SO_4 above its eutectic point. When ready for use, 0.1 cc. plasma is measured into each of nos. 1, 2 and 3, and 0.2 cc., into nos. 4 and 5, the volumes made up to 10 cc. with warm water and the flasks returned to the incubator for three hours.

Composition of buffer (*Berglund*[1]): 29.6 cc. of 0.2 M carbonate free NaOH, 50 cc. 0.2 M of KH_2PO_4, distilled water (carbonate free) to 100 cc. This should give a solution with pH 7.0.

At the close of the three hour period, the contents of flasks nos. 2, 3, 4 and 5 are filtered. A fine smooth filter paper 6 cm. in diameter is employed. The filtrates are collected in graduated centrifuged tubes; 9 cc. can usually be procured. At the same time 9 cc. of the contents of flask no. 1 are transferred to centrifuge tube no. 1. To each of the five tubes is added 1 cc. of 50 per cent trichloracetic acid and the contents thoroughly mixed by means of a fine glass rod which is then washed off with 4 per cent trichloracetic acid. The centrifuge tubes are allowed to stand for ten minutes at room temperature, and are immersed for ten minutes in a water bath at 50°. They are centrifuged at 3500 revolutions per minute for twenty minutes, after which the supernatant liquid is decanted and the precipitate dissolved in one or two drops of 10 per cent NaOH stirring thoroughly with a fine glass rod and spreading the NaOH over the entire inner surface of the centrifuge tube. The contents are then made up accurately to the original volume (9 cc.).

The nitrogen of each of these solutions is determined by Folin's micro kjeldahl method, employing 2 cc. of each. Occasionally 3 cc. may be required for some of the lower fractions of globulin.

$$\textit{Computation:}\ S \times \frac{20}{R} \times \frac{10}{X} \times \frac{100}{Y} = N$$

where S = value of standard, R = reading of colorimeter, X = 0.1 or 0.2 cc., according to the fraction being ashed, Y = no. of cc. ashed, and N = milligrams protein nitrogen in 100 cc. of plasma. To obtain the grams of protein per 100 cc. of plasma, multiply by 6.25 and divide by 1000. Total protein equals protein in tube 1; fibrinogen equals tube 1 minus tube 2; euglobulin equals tube 2 minus tube 3; pseudoglobulin I equals tube 3 minus tube 4; pseudoglobulin II equals tube 4 minus tube 5; albumin equals tube 5. In case values of only total protein, fibrinogen, globulin and albumin are desired tubes 3 and 4 are omitted.

Table for Computing Protein in Plasma

(Grace Medes and Lloyd Medes)

Grams of protein in 100 cc. plasma, computed from formula $0.15 \times \frac{20}{R} \times \frac{10}{0.1} \times \frac{100}{2} = N$ when 0.15 is value of standard in milligrams; R is reading of colorimeter with standard set at 20; when 0.1 cc. of plasma is diluted to 10 cc., and 2 cc. of the filtrate are ashed for nesslerization.

R	N	R	N	R	N	R	N	R	N
12.1	7.75	15.1	6.21	18.1	5.18	21.1	4.44	24.1	3.89
12.2	7.68	15.2	6.17	18.2	5.15	21.2	4.42	24.2	3.87
12.3	7.62	15.3	6.13	18.3	5.12	21.3	4.40	24.3	3.86
12.4	7.56	15.4	6.09	18.4	5.10	21.4	4.38	24.4	3.84
12.5	7.50	15.5	6.05	18.5	5.07	21.5	4.36	24.5	3.83
12.6	7.44	15.6	6.01	18.6	5.04	21.6	4.34	24.6	3.81
12.7	7.38	15.7	5.97	18.7	5.01	21.7	4.32	24.7	3.80
12.8	7.32	15.8	5.93	18.8	4.99	21.8	4.30	24.8	3.78
12.9	7.27	15.9	5.90	18.9	4.96	21.9	4.28	24.9	3.77
13.0	**7.21**	**16.0**	**5.86**	**19.0**	**4.93**	**22.0**	**4.26**	**25.0**	**3.75**
13.1	7.16	16.1	5.82	19.1	4.91	22.1	4.24	25.1	3.74
13.2	7.10	16.2	5.79	19.2	4.88	22.2	4.22	25.2	3.72
13.3	7.05	16.3	5.75	19.3	4.86	22.3	4.20	25.3	3.71
13.4	7.00	16.4	5.72	19.4	4.83	22.4	4.19	24.4	3.69
13.5	6.94	16.5	5.68	19.5	4.81	22.5	4.17	25.5	3.68
13.6	6.89	16.6	5.65	19.6	4.78	22.6	4.15	25.6	3.66
13.7	6.84	16.7	5.61	19.7	4.76	22.7	4.13	25.7	3.65
13.8	6.79	16.8	5.58	19.8	4.73	22.8	4.11	25.8	3.63
13.9	6.74	16.9	5.55	19.9	4.71	22.9	4.09	25.9	3.62
14.0	**6.70**	**17.0**	**5.51**	**20.0**	**4.69**	**23.0**	**4.08**	**26.0**	**3.61**
14.1	6.65	17.1	5.48	20.1	4.66	23.1	4.06	26.1	3.59
14.2	6.60	17.2	5.45	20.2	4.64	23.2	4.04	26.2	3.58
14.3	6.56	17.3	5.42	20.3	4.62	23.3	4.02	26.3	3.56
14.4	6.51	17.4	5.39	20.4	4.60	23.4	4.01	26.4	3.55
14.5	6.47	17.5	5.36	20.5	4.57	23.5	3.99	26.5	3.54
14.6	6.42	17.6	5.33	20.6	4.55	23.6	3.97	26.6	3.52
14.7	6.38	17.7	5.30	20.7	4.53	23.7	3.96	26.7	3.51
14.8	6.33	17.8	5.27	20.8	4.51	23.8	3.94	26.8	3.50
14.9	6.29	17.9	5.24	20.9	4.49	23.9	3.92	26.9	3.49
15.0	**6.25**	**18.0**	**5.21**	**21.0**	**4.46**	**24.0**	**3.91**	**27.0**	**3.47**

This method has been checked against the corresponding macro method in which 1 cc. of plasma is used for each fraction, and found to check within the limits of error of the macro method.

SUMMARY

A micro method for estimation of the protein fractions of plasma is described. For complete fractionation 0.7 cc. of plasma is required. For fractionation into fibrinogen, albumin and globulin, 0.4 cc. of plasma is used.

The method depends upon fractional salting out with anhydrous Na_2SO_4, precipitating the protein remaining in the various filtrates with trichloracetic acid, dissolving the precipitates in NaOH and determining the nitrogen in an aliquot part.

The error of the determination is not greater than that of the corresponding macro method.

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GELATINOUS CARCINOMA OF THE BREAST

Second Report

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In a previous communication[1] on this subject it was regreted that the case reported at that time did not permit a description of the histology of the metastases. For that reason the following case report is considered important.

CASE REPORT

A radical amputation of the left breast was performed on a woman, aged 59, January 11, 1933. The clinical history revealed that three months prior to operation the patient was struck over the left breast; the blow was not severe. Several weeks later she noticed that the nipple was firm and appeared to be retracted. Subsequent to this she noted a small growth in the left breast which she treated with poultices. This growth progressively enlarged and soon was the seat of severe throbbing pain. Shooting pains into the left axilla were noticed upon movement of the left arm. There has not been any discharge from the nipple. There was no loss of weight. Past history, previous illnesses, family history were of no consequence.

Physical examination revealed that the right breast was free of palpable tumor masses, but that a hard, irregular, tender mass was present in the outer, lower quadrant of the left breast close to the nipple. It was not freely moveable. The nipple was retracted, not discharging. The skin over the tumor was somewhat reddened and nodular. There was a large, tender gland in the left axilla about the size of a walnut. The pre-operative diagnosis was: carcinoma of the left breast with extension to the axilla.

Pathological Report: "The specimen consists of the left breast, pectoral muscles and axillary fat tissue. The nipple is depressed, slightly bluish in color. There is a crescentic elevation around the margin of the nipple, so that the normal groove between the nipple and skin is effaced. Section through the longitudinal axis in the nipple line reveals a non-encapsulated, distinctly colloid or gelatinous tumor about 2.5 cm. in diameter. It is located in breast tissue and extends upward into the nipple zone. This upper portion is more distinctly

of a tumor character, homogeneous, gray tissue, quite lacking in colloid material. The colloid structure of this tumor is so striking as to warrant the descriptive term "honey-comb." In the axillary fat a large nodule of similar tissue is found, cross section of which reveals a distinctly neoplastic, gelatinous-filled structure. The microscopic examination reveals a cellular, rather anaplastic, papillary growth, in which there are numerous mitotic figures and irregular invasion of the stroma (Figure 1). In the colloid areas the cells are disrupted and atrophic. One may trace the origin of the colloid material in both the axillary mass and the

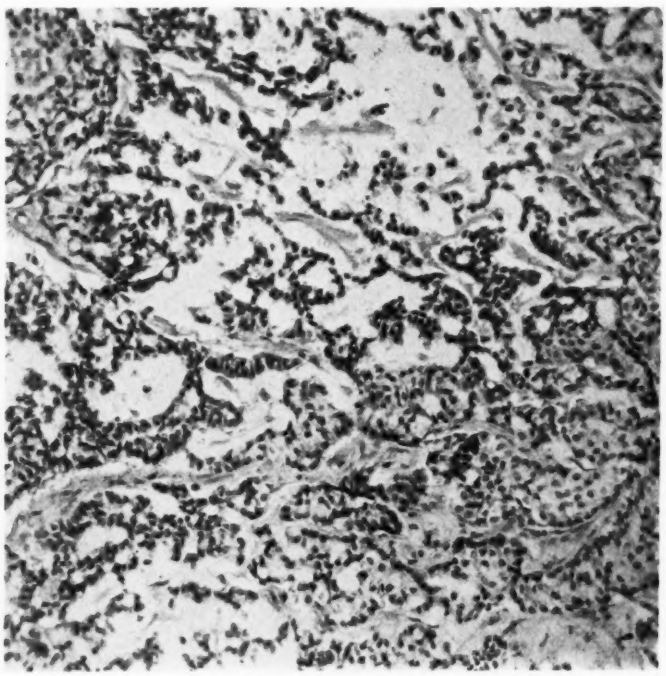

FIG. 1. METASTATIC AXILLIARY LYMPH NODE

primary tumor to the cells themselves. The cytoplasm seems to have filled up with this material to the point of cell rupture, whereupon the stroma was filled with this material and the cells either destroyed or so compressed as to be hardly distinguishable."

This case illustrates the mechanism of colloid change in carcinoma of the breast and offers striking proof that the process is epithelial in origin. The nature of this process would seem to be

one of intracellular metabolism. Gelatinous carcinoma of the breast should be considered as possessing peculiar and destructive qualities which are carried with the cells and reproduced in the metastases.

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A SIMPLE METHOD WITH A NEW APPARATUS FOR RAPID DIALYSIS*

Preliminary Report

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P. LeComte du Noüy[6] reached an interesting conclusion from his experiments on the surface tension of proteins which he states as follows:

> If we attempt to calculate the size of the vessels which would require pure serum to develop such a monolayer . . . . it is found that they would have to be of the order of magnitude of the blood capillaries, filled with blood cells. Thus one of the factors governing the concentration of colloids in the serum is perhaps found."

This conclusion led one of us (I. A. N.) to speculate on the possibility of studying fresh uncoagulated blood in vessels approaching the capillary size, or the whole blood with its proteins approaching the monolayer state. No way to make capillary tubes seemed practical so the possibility of spreading the blood very thin was tried. No monolayer studies are reported at this time but experiments indicate that this apparatus might be of practical use in routine procedures in medical, industrial, and research laboratories. The data included in this report are merely for the purpose of illustrating the rapidity and reliability of the apparatus.**

It was found that by putting 0.2 cc. of blood on the convex surface of a large watch glass and spreading it out by means of a

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

** At the author's suggestion, the Central Scientific Company is considering the development of a suitable commercial model.

piece of wet cellophane stretched tight on an embroidery hoop 17.8 cm. (7 inches) in diameter, a spread of 15.3 cm. could be obtained. Calculations showed that the thickness of the blood layer was about 6 microns. It is evident that the total surface achieved (area of spread plus the area of all the erythrocyte surfaces) enables one to approach a monolayer state of the proteins much closer than with a collodion sac or similar dialyzer.

Tests for blood sugar at this time showed (by Folin's ferricyanide method) that in two minutes dialysis and equilibrium were complete when using 0.2 cc. of blood, 9.8 cc. of water and a dry cellophane.

The tests were repeated with larger frames and larger amounts of water and varying amounts of blood. When 4 cc. of serum with a spread of about 20 cm. and 46 cc. of distilled water were used, equilibrium was almost complete in twenty minutes. These tests showed roughly the relationship of surface, blood thickness, amount of diluent, and time in attaining an equilibrium with this apparatus. They also showed that by choosing proper curvatures a relatively large amount of blood could be held in contact with the dialyzer, even though the blood was not in a retaining vessel.

Up to this time rubber bands had been used to hold cellophane on the frame. Sometimes the rubber bands held so firmly that the cellophane cracked in drying. Also, on rewetting, the cellophane tended to sag and the slack had to be taken up by gently pulling on the edges. It became apparent that some way would have to be devised whereby cellophane could be held firmly stretched when wet and yet not crack when dried on the frame.

The following prerequisites were found necessary:

(1) The wetted cellophane must be fastened with enough sag to allow for a difference of from 2.5 cm. to 4.0 cm. for each 30 cm. in expansion and contraction when in the wet and dry states.

(2) The cellophane should not dry in contact with rubber or any other surface except at the circumference because it tends to adhere and when wet again, to crack. Even if no cracking occurs it tends to peel off a scum from the rubber making the dialysate solution turbid.

(3) Visibility of the colloid must be maintained to insure uniform spreading during manipulation of the apparatus.

Various forms of apparatuses were devised which would overcome these difficulties. In addition such accessories as vacuum and pressure control, temperature regulation, continuous operation, and electrodialysis were devised. But finally special efforts were made to construct a simple model which any technician could assemble and use with a minimum of expense and mechanical ingenuity. Since it is not necessary to have a dried cellophane for all routine work, two models are suggested.

DESCRIPTION OF APPARATUS FOR DRIED CELLOPHANE

A model for the use of cleaned and dried cellophane requires and is made as follows:

(1) A grooved rim on which to fasten a continuous rubber tube and the cellophane. A metal wheel about 24 cm. in diameter and 0.6 to 1.3 cm. wide (such as come on baby carriages) is satisfactory.

(2) A continuous rubber tube with an air vent is formed over the above rim. (The Miller Rubber Products Company made some rubber bladders about 21 cm. wide and 25 cm. long, with a vent on the 21 cm. side—such as they make on rubber toys.) The 25 cm. sides are open or should be trimmed so one has in effect not a bladder but a large open rubber tube. Stretch one open end evenly over the above rim and tie on with a string. Now overlap the other end of the tube over the first end so that the rim is entirely within a continuous tube with the vent 2.5 cm. from the edge. When the rubber is so adjusted that it is even, with a central hole about 18 cm. in diameter, tie firmly. A little rubber glue may be smeared between the two layers of rubber by reflecting the overlap so a space over the rim and into the groove may be reached. The finished frame now looks like a flattened rubber doughnut which can be inflated. The continuous rubber tube enables one to allow a sufficient expanse of wet cellophane so it will not crack when dry and provides a mechanism for holding it stretched when wet again by simply inflating.

(3) A base is used on which to place the colloid solution. Any object with a convex surface may be used. We have used large watch glasses and lids from kitchen utensils. These may be paraffined. However, much more control may be obtained if the convexity can be adjusted. We have found that a rubber surface does not interfere with the blood tests so far done except possibly in the case of calcium. We are using a 25 cm. cake pan with a sheet of rubber stretched over the top and tied over the edge. An air vent is soldered into the side of the pan.

(4) A cover is convenient to keep out dirt and lessen evaporation if one is interrupted and has to let the set up stand for some time. A large watch glass, large funnel, or inverted vessel may be used.

To fasten the cellophane on the frame the tube is inflated to about 5 cm., the cellophane thoroughly wetted and carefully smoothed over the groove of the apparatus. If a small rubber band is slipped over the cellophane and into the groove, the cellophane may be easily smoothed of any sharp wrinkles as it seems to be somewhat plastic when wet. The cellophane may be fastened on firmly by using a sufficient number of rubber bands, or tied on. A rubber band as wide as the rim may be used and the string tied over this and thus remain dry. All excess cellophane should be trimmed close to the tie as cellophane tends to act as a wick, introducing possible contamination from the outside or loss of some dialysate solution.

To dry the cellophane the rubber tube is deflated completely. This draws the rubber away from the plane of the cellophane thus allowing it to be kept dry for any length of time without sticking to the rubber. While drying, the dialyzer may be suspended by clips fastened to the excess overlap.

METHOD OF USING THE DIALYZER

(1) Place the dialyzer on the base for convenience in handling and put the dialyzing fluid in the dialyzer. Distribute it so that all the cellophane becomes thoroughly wet in a short time with less evaporation.

(2) Now inflate the rubber tube until the cellophane is stretched.

(3) Inflate the rubber drum of the base and place the colloidal solution to be analyzed on the dome. It will not run off.

(4) Gently lower the dialyzer on the colloidal solution. By raising and lowering, or rotating the dialyzer, the colloidal solution may be teased to wet the cellophane in wider circles in a few seconds. The slower the blood is spread, the thinner will be the layer, just as in making blood smears for differential counts. The drum may now be deflated until plane and thus get an even distribution of the dialyzing solution in the dialyzer. The dialysis seems to reach rapid equilibrium without further manipulation.

If a dialysis is desired and the dialyzer is not dry, it may be washed and used in a manner to be described under the wet method.

DESCRIPTION OF A METHOD FOR USING WET CELLOPHANE

All that is needed is a bell jar of the Graham type or a suitable frame on which to tie the cellophane. Thoroughly wet a sheet of cellophane and stretch it smoothly over the frame, tying with string or using several rubber bands. Wash and drain off the excess water. A 25 cm. dialyzer takes up roughly about 3 cc. of fluid, so if a 1 in 20 dilution is wanted, add 16 cc. of fluid when 1 cc. of colloidal solution is used. Dialyze as with the dry method, manipulating the coaptation of the cellophane to the surface of the base with the colloidal solution between them. After equilibrium is established, remove a measured quantity of the dialysate for the determinations and add a measured quantity of a colored dialyzable solution such as alkalinized phenolphthalein from a stock on

hand. After equilibrium is reached with this (ten minutes seems sufficient), remove some of the now colored dialysate in the dialyzer and compare in the colorimeter against the stock colored solution. Calculate the water originally present in the cellophane. After using this dialyzer, it may be washed and set in some appropriate space in the laboratory, with water in the dialyzer to avoid drying and breaking of the cellophane. Recent preliminary tests indicate that a few drops of glycerol spread out on the wet cellophane keep it moist for weeks without cracking and inhibit the growth of fungi.

REPORT ON SOME EXPERIMENTS

A patient was admitted to the Hospital with very high non-protein nitrogen blood constituents. We had found it more satisfactory and accurate to use from 19 cc. to 49 cc. of water in a 25 cm. dialyzer to 1 cc. of blood on the base. These ratios

TABLE 1

COMPARISON OF VALUES

(Mgms. per 100 cc).

METHOD	NON-PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE
Dialysate	190.0	141.5	8.1	13.5
Filtrate	193.0	141.5	7.9	13.5

permitted us to use our routine reagents for the high values for non-protein nitrogen substances in this patient's blood. For the regular determinations the filtrates were diluted accordingly. The precipitations were started at the same time as the dialyses. Ten minutes were allowed for the dialyses to reach an equilibrium. The same standards were used for the dialysates and the filtrates (see table 1).

These and other tests have convinced us that this dialysate method is at least as fast and as accurate as precipitation and filtration for the separation of free crystalloids from the colloids of the blood.

GENERAL REMARKS

The apparatus is readily washed. Soak the cellophane and base with a weak sodium bicarbonate solution, rinse and dry.

Soap may be used. Gortner[2] quoted Brinkman and Szent-Georgyi to the effect that sodium oleate will make a collodion sac permeable to hemoglobin at three atmospheres pressure. We have found no significant alteration in permeability in ten minutes with cellophane that had been washed with ivory soap and that had been rinsed thoroughly.

We have used Congo red to test for leaks. Michaelis[4] reported that protein may take Congo red through an ultrafilter which (due to surface charge) would not permit Congo red alone to pass through. We have left Congo red mixed with serum on the dialyzer for hours and could not detect any Congo red color in the dialysate from cellophane # 300, even after concentrating a quantity of dialysate. Starch has been used by Aitken[1] to test the permeability of collodion sacs. We have left starch on the dialyzer for hours and could not detect any color in the dialysate with the iodine test.

Cellophane # 300 has been used because it has been tested by various workers and found to be impermeable to proteins. Nicholas[5] used 200 lbs. pressure for one and three-fourth hours and found no proteins in the ultrafiltrate. Cellophane is readily obtained, is uniform, is cheap, and we have found that by the method presented in this paper it may be used repeatedly. However, the apparatus and methods are adaptable to other membranes which might be used or developed with a consideration for physical or chemical factors.

Freshly drawn whole blood has been used without anticoagulants, in some tests. It appears that the free calcium is lost so rapidly that together with a certain amount of defibrination which seems to occur, no coagulation takes place.

Most of the laboratory manuals on colloid chemistry describe various types of dialyzers. Some of these exhibit a large amount of surface with a small amount of liquid to be dialyzed—such as Pauli's folded parchment paper.[3] There have also been a number of methods reported in recent years using collodion sacs for rapid dialyses. So far we have not found any apparatus using cellophane or other membrane repeatedly in an apparatus or method such as I have presented.

SUMMARY

Two models of an apparatus using cellophane #300 and a method of obtaining certain blood dialysates at least as rapidly and accurately as blood filtrates, have been described.

It is possible to spread blood with its erythrocytes in such a thin layer with this apparatus that the proteins probably approach a monolayer state.

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REMOVAL OF THE SPINAL CORD BY THE ANTERIOR ROUTE: A NEW POSTMORTEM METHOD

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Removal of the spinal cord and its membranes by reflecting the skin and muscles of the back, and removing the laminae and spinous processes of the vertebrae has been a laborious task, and has retarded satisfactory study of this structure and its appendages. The modification of this technic which is the subject of this note has been used in the laboratories of Pathologic Anatomy of The Mayo Clinic for eight years, and has been so satisfactory and comparatively simple that it seemed to me to be worth recording.

No elaborate equipment or instruments are necessary, and no new ones are required. A saw with a rounded end, a wooden hammer, and an all steel chisel are used. Part of the standard equipment is a large pair of Councilman's bone cutting forceps* used to remove the inner and middle ears, and formerly used extensively by orthopedists.

After the organs have been removed from the thorax and abdomen, the bodies of the vertebrae are cleaned, and then cut with the saw in the direction indicated in figure 1. The cutting should be commenced at the promontory of the sacrum and then continued upward to the fourth thoracic vertebra or even higher. The pedicles on the right are cut, either at the level of the intervetebral foramina with the chisel, or with Councilman's forceps, and leverage is obtained to remove the segment of vertebra with the large forceps. If assistance is available, a chisel inserted into the cut also pries the segment of vertebra laterally, and assists in its removal. The half body of the vertebra can be re-

* Councilman's Bone cutting Forceps No. A/-300, obtained from The KNY Schierer Company, New York.

moved individually by cutting the intervertebral disks with a knife; this is a simple procedure, and usually advisable; it permits also examination of the cartilage of the intervertebral disks.

When the segments of vertebrae are removed, the anterior surface of the dura mater and the nerve roots (fig. 2), as well as the dorsal root ganglion and nerve trunks, are exposed. The dura mater with the spinal cord within, the nerve roots, and dorsal root ganglia can be removed intact by cutting the nerve roots of the left side, or if desired, the dorsal root ganglia on this side can be easily obtained for examination. The only objection to this method is the possibility of injuring the spinal cord with the saw, but after a little experience, this danger disappears. Another

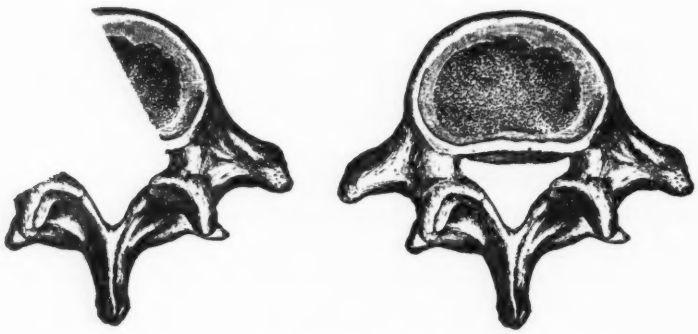

FIG. 1. AMOUNT OF EXPOSURE WHICH CAN BE OBTAINED

possible objection is that the cervical segments of the spinal cord are not exposed or readily obtained, but if the cervical cord is desired a thin knife can be inserted between the occipital bone and the atlas and the medulla cut across. The dura mater at its upper level of exposure is cut completely around, and with gentle traction toward the feet, the entire cord, without the dura mater and dorsal root ganglia can be obtained. The spinal cord secured in this manner is intact and satisfactory for histologic or chemical studies.

The advantages of this method compensate for any possible objections which might be made to it. Special permission to examine the spinal cord is not necessary, special equipment is not

necessary, the body does not have to be turned, and there is less work for the undertakers. Sufficient strength of the vertebral column remains, but if desirable, the defect in the bones can be

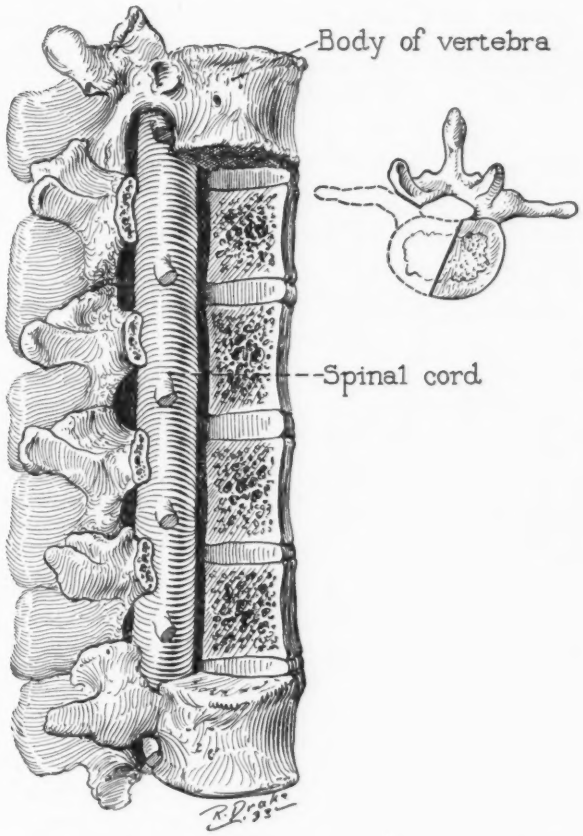

FIG. 2. EXPOSURE OF THE SPINAL CORED SURROUNDED BY THE DURA MATER AS WELL AS THE NERVE ROOTS

The dorsal root ganglia and the nerve trunks can also be removed completely. This exposure can be obtained for the entire spinal cord up to the third thoracic vertebra or even higher.

repaired with plaster of paris. By this method the work can be done more easily and more quickly than by that ordinarily used. In this manner I have obtained for examination several hundred

spinal cords, and Harmeier has used the method exclusively when removing the filum terminale for his study of the normal structure of this tissue. The method is particularly advantageous in obtaining the lumbar portion of the spinal cord, as well as the cauda equina. At this level, it is difficult to approach the spinal cord from the back because of the heavy muscles, the sacro-iliac joint, and the depth of the cauda equina from the surface.

TORULA MENINGITIS*

Report of Case and Summary of Literature

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Medical knowledge of diseases due to parasitic plants is astonishingly recent in origin, the discovery of such minute objects necessarily awaiting the advent of optical magnification. Fungi, being larger than bacteria, were naturally discovered first. Dr. Robert Hooke, of England, using his bubble of glass filled with water as a microscope, in 1656 described a fungus disease of rose leaves, but not until the advent of the compound microscope of two hundred years ago was it possible for Langenbeck to describe the yeast-like cells found covering the oral and pharyngeal mucosa of a cadaver at autopsy, and for Charles Robin to accurately describe "thrush" as a fungus disease in 1843.

Fungi approach and often exceed the size of the average body cell. They are not able to grow and reproduce within the animal body to the unlimited extent that bacteria can. They produce a relatively small amount of exotoxins. Their disease processes are more probably due to mechanical displacement of cells thereby altering normal physiologic processes. For these reasons fungus diseases are usually characterized by great chronicity and very slight infectivity. Probably all of the systemic fungus diseases represent very accidental implantations of microscopic plants which are barely able to survive and reproduce slowly within living animal tissues.

Out of a total of something over 5000 genera of fungi recently listed by Clements and Shear[1] it is significant that there are only twenty-six well recognized groups that can and do produce definite

* Read before the Twelfth Annual Convention of the American Society of Clinical Pathologists, Milwaukee, Wisconsin, June 9 to 12, 1933.

disease processes. It is highly improbable that there will be many additions to this list. With the exception of members of the order of Trichomycetes, or "higher bacteria," as many medical writers prefer to call them, medical knowledge is fairly complete in its conception of etiology and pathology. Nomenclature, or rather a superabundance of it, has served to produce a state of utter lack of specific information about these few diseases in the minds of not only medical practitioners but the authors of the majority of our medical laboratory text-books as well. For this reason alone the recognition of systemic fungus infections by otherwise competent physicians and clinical pathologists has just about reverted to the par value of one case per lifetime which was established by Charles Robin some two hundred years ago.

CASE REPORT

Mr. D. L., white male, aged forty-eight years, book-keeper, consulted Drs. Y. Ardoin and C. L. Attaway, of Ville Platte, Louisiana, on Feb. 2, 1932, for relief from a persistent headache. The patient had had recurrent attacks of migraine for a long period and insisted that this particular headache was different, describing it as a "bursting headache," not affected in the slightest by any of his usual remedies. The usual regimen with catharsis and diet gave no relief.

Within two weeks drowsiness, blurred vision, loss of weight and strength were definitely present. He continued work for the next three weeks, finally entering the hospital on March 17.

Complete examination upon his entry into the hospital showed an emaciated patient with slow and interrupted speech. The general physical findings were as follows: a slight but definite cervical rigidity; positive Kernig; tendon reflexes increased generally; negative findings in chest and abdomen; a small granuloma with crusted surface in the right submaxillary region, which was said to have developed from a razor cut; temperature, 99°F., pulse 60, respiration 18; roentgen ray examination of skull, stomach and chest were negative.

The patient alternated between periods of extreme restlessness and prolonged stupor, with eyes closed practically all of the time. When aroused he would only ask for something to relieve the headache, which was only accomplished by the use of morphine.

On March 25 the spinal fluid examination revealed a meningitis caused by a fungus, which we will describe more in detail later. Scrapings from the lesion on the jaw revealed the same type of budding yeast-like fungus found in the spinal fluid. The spinal fluid findings were re-checked on subsequent lumbar puncture for therapeutic relief of pressure.

The patient gradually lost ground, his pulse remained slow and his tem-

perature was usually subnormal. He died on April 10, 1932, following an illness of seventy-three days.

An autopsy was denied. The lesions illustrating the following discussion were produced in rabbits by cisternal injection of the pure cultures obtained from the spinal fluid.

DISCUSSION

Systemic infections by a pigment producing yeast that reproduced by budding only, on living or dead media was described as a clinical entity by Stoddard and Cutler,[4] of Boston, in 1916. The fungus was determined as a member of the Torula group by these workers and named *Torula histolytica* from the characteristic appearance of disintegrated brain tissue surrounding colonies of the fungi. In addition to their two cases and the four cases that they were able to identify in previous literature, we have attempted to obtain a complete list of systemic torular infections by reviewing all apparently pertinent titles appearing in the Cumulative Index of Medicine. Including this case the total number of authentic systemic infections due to torula has reached a total of forty-six. Of this number thirty-one have occurred in the United States and fourteen have been recorded from England, Germany, Australia, Japan, France, Dutch East Indies and Italy. The organism thus apparently lives independently of man, and is world-wide in its distribution.

Clinically, human infections with yeasts of the genus Torula usually involve the central nervous system and by preference the meninges (pia and arachnoid). A few instances are on record of small abscess-like areas within the gray matter, or involving only the lungs. The organisms gain entrance into the body chiefly by way of the respiratory tract, although the portal of entry is often not ascertainable. In one instance trauma over the scapula was followed by a deep mycotic infection that had healed many months before the onset of the typical meningitis. This is in direct contrast to systemic infections with *Cryptococcus gilchristi* (blastomycosis) or *Coccidioides immitis*, where the primary lesion almost invariably occurs in the skin. In our present case the initial lesion was proved to be a small superficial granuloma that developed from a razor cut.

The lesions of torulosis will not be found described in any of the texts commonly employed in pathology. Stoddard and Cutler's original description of the two types of central nervous involvement (meningeal or focal within the gray matter of either the cerebrum or the cerebellum) will be found most complete. Freeman[2] has more recently reviewed this pathology in a survey of all of the various fungous lesions involving the brain or its coverings. His description is complete and well illustrated.

Essentially the torular lesions are chronic inflammatory in type, the organisms consisting of colonies of yeast-like budding fungi lying in areas of liquid or necrotic material. Such a characteristic lesion was shown in rabbits inoculated with cultures from our case which was of the meningeal type (see fig. 4). The organisms present a characteristic zone of retraction (or are surrounded by a clear transparent secretion, as this is occasionally described). The smaller fungi stain rather darkly with Gram's stain, and the larger ones present a definitely stained, thick cell wall with a dark central mass situated rather eccentrically. The appearance of torula is quite characteristically different from the *Cryptococcus gilchristi* (see fig. 6), which has a thick non-stained cell wall, with several dark-staining masses of nuclear material within the body of the fungus, and neither of these resembles the endosporulating type of *Coccidioides immitis* (figs. 7 and 8). All of these illustrations were photographed with the same optical system.

FIGS. 1 TO 8

FIG. 1. Spinal fluid showing type of cells (mononuclears) and fungi (*Torula histolytica*) present.

FIG. 2. Budding forms of fungus found in fresh fluid.

FIG. 3. Multiple budding shown in microcultures.

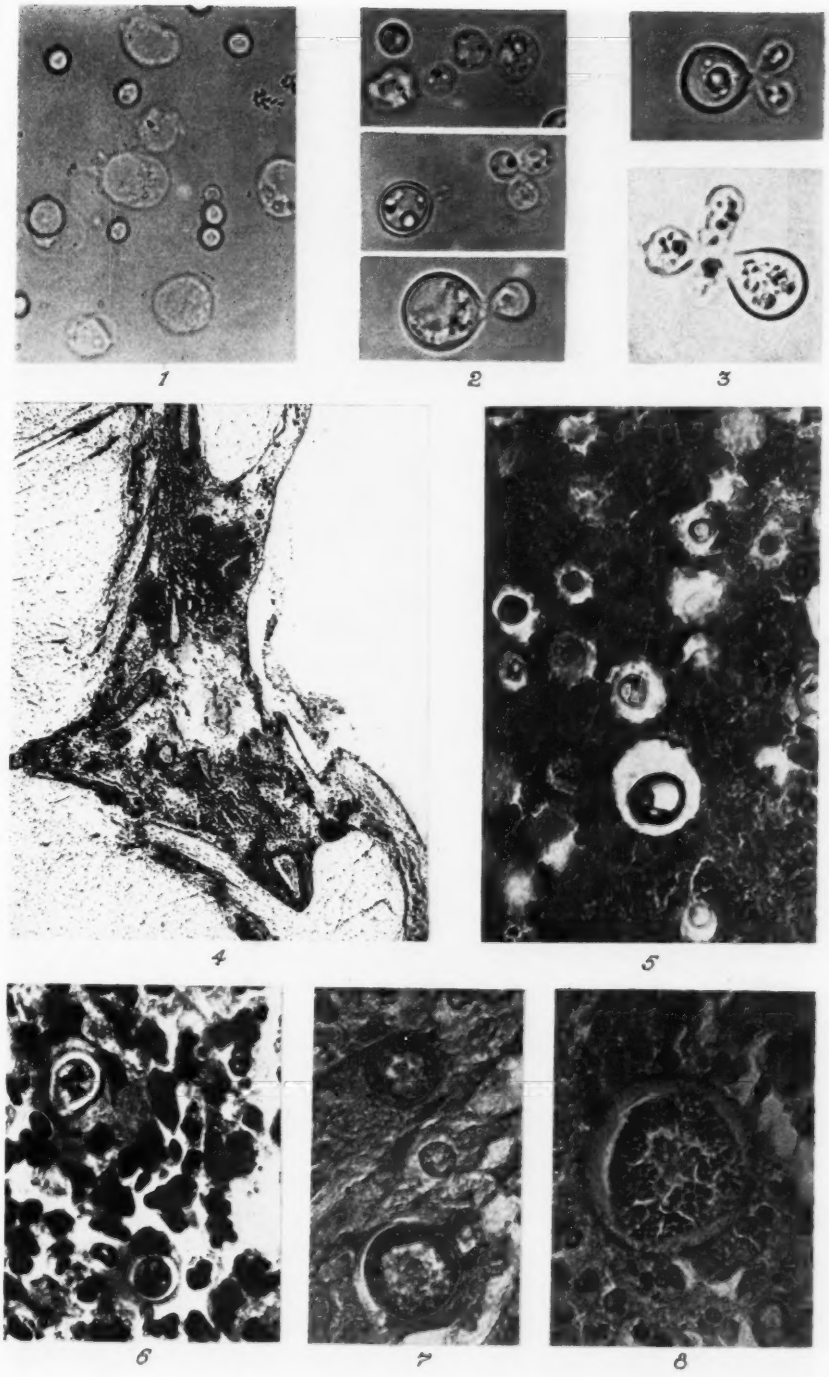
FIG. 4. Chronic inflammatory reaction with edema in the meninges. Dark area is a collection or colony formation in necrotic material shown in Fig. 5.

FIG. 5. *Torula histolytica* present in necrotic material in meningeal lesion (rabbit).

FIG. 6. *Crytococcus gilchristi* in lesion of lung.

FIG. 7. *Coccidioides immitis* in lesion of lung.

FIG. 8. Eudosporulating form of coccidioides in lung.



FIGS. 1 TO 8

While the great majority of torular lesions have been frankly meningitic in type, only a very few cases have been diagnosed before death by means of cerebrospinal fluid examination. There is a still shorter list of cultural studies of the organism. The spinal fluid of our case was faintly hazy by indirect light, with 650 mononuclear cells and a slightly larger number of torula cells (see fig. 1). The common practice of using the 16 mm. objective for cell counting has undoubtedly contributed largely to the lack of recognition of the infection. Under higher magnification (1/12 oil immersion objective, No. 4 Leitz ocular and a camera bellows of 18 inches, which was also used in photographing figs. 5, 6, 7 and 8) the thick walled budding fungus may be recognized with certainty. The thick, highly refractile cell wall of the larger forms resembles cryptococci (blastomycetes). Microcultures in glucose veal infusion agar in our case soon revealed characteristic multiple budding forms without mycelia. Yellowish pigment production was noticeable on surface agar colonies within a week, during which time none of the common sugar mediums was appreciably fermented. Scrapings from the lesion on the jaw yielded a morphologically identical budding fungus.

Several reported cases have been undoubtedly produced by a non-pigment-forming type of torula. This calls for some further classification of species. Applying Harrison's[3] classification of the torulae to the most correctly employed botanical nomenclature of Clements and Shear[1], we may classify these fungi as follows:

Phylum, Thallophyta; Sub-division, Eumycetes;
Group, Deuteromycetes (Hyphomycetes or Fungi Imperfecti)
Order, Moniliales; Family, Pseudosaccharomyces;
Genera, Rhodotorula, producing red pigment.
Chromotorula, producing other pigment.
Torula, producing no pigment.

The common name for the fungus discussed in this paper as now used throughout the medical literature is *Torula histolytica*, Stoddard and Cutler, 1916. Unfortunately the designation of *Torula Histolytica* was given by Harrison to one of the very few non-chromogenic cultures of human origin. Much valuable

literature would be lost to students of medicine if teachers and pathologists discard this name.

We should prefer to see some large group of clinical pathologists correlate the commonly known names of the pathogenic fungi into an orderly medical classification and describe accurately and illustrate their undoubted characteristics in tissue or standard cultures. Then by means of a relatively small list of synonyms the medical worker could transfer his findings to some such botanical classification as is attempted by the Saccardo system used extensively abroad, or its interpretation by our own American botanists, Clements and Shear. Until such time as will witness the ability of the average clinical pathologist to determine with a fair degree of certainty such fungi as are commonly found in various human exudates, the number of such cases that escape proper recognition can only be conjectured. It is certain, however, that only a fraction of the actual incidence of such infections is represented by the small number of such cases that appear in the records.

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EDITORIAL

Viruses—A New Era in Medicine

The history of modern medicine may be divided into three periods each of which is characterized by the dominant part played by a particular laboratory group. First the morphologists, to whom we are indebted for rescuing medicine from what Garrison calls "fanciful speculation," held the center of the stage until the creation of the wholly new science of bacteriology. Workers in this new field of research contributed so much to the knowledge of the cause of the infectious diseases and to the prevention and cure of some that they compelled the attention of the medical world for many years but were in turn superseded by the bio-chemists who had long been busy laying the foundations for the wonderful discoveries now familiar to all.

There are indications that we have entered a fourth period which is to be dominated by still another laboratory group namely, the investigators of the filterable viruses. These men have shown such imagination, energy, and ingenuity that one does not hesitate to predict that their investigations will result in discoveries comparable to those of the bacteriologists and bio-chemists. Space limitations permit the mention of only a few examples of their work.

Li and Rivers[8] have demonstrated that some of the viruses can be grown in large quantities in a medium of minced chick embryo and Tyrode Solution whereas until recently the only method available was tissue culture. Goodpasture and his associates[5] at Vanderbilt University have contributed a brilliant and prize-deserving method in which the chorio-allantoic membrane of chick embryos is used as a medium. They have not only grown the viruses of vaccinia, fowl-pox, and herpes simplex but have studied the reaction of the three types of embryonic cells to them. Stevenson and Butler[12] following Goodpasture's sug-

gestion and using his method have succeeded in growing vaccine virus in large quantities and free from contaminating organisms and claim superiority of the method over that customarily used. Burnet[2] has employed the same method in the study of canary-pox. The Vanderbilt group[13] has proved that the virus of fowl-pox is in the cell inclusions associated with the lesions and have gone even further in that they have produced the disease by injecting some of the minute elementary bodies which are found within the inclusions.[14] Amies[1] and Ledingham[7] have shown agglutination of the elementary bodies of vaccinia and varicella by the serum of patients with these diseases and Parker and Muckenfuss[9] have performed complement fixation reactions in vaccinia and variola using as antigen the contents of pustules which are known to contain the elementary bodies in large numbers. So much evidence has been brought forward to prove that these bodies cause disease that Goodpasture[4] has proposed the name Borreliota as a generic title and a variety name for each strain thus not only giving a name to these interesting structures but also doing honor to Borrell their discoverer.

While Borreliota have been found in many of the virus diseases there are many others in which no particulate matter has been demonstrated, nevertheless much interesting work is being done with these too. Shope[10] has obtained a filterable virus from swine suffering from swine influenza which, in combination with a hemophilus bacillus, produces the disease when applied to the nasal mucosa of healthy animals. Using the virus in the same way but without the bacillus or by injecting it intramuscularly the disease is not produced but a solid immunity is conferred. Smith, Andrewes, and Laidlaw[11] have recovered a virus from patients with human influenza which shows a close antigenic relationship to the swine virus. Ferrets are equally susceptible to the two viruses and the serum of an animal recovered from the inocculation of one will protect against the other.

Still another group of diseases is being investigated by the virus workers. Furth[3] has produced leukomatosis, myelomatosis, a variety of endothelioma, and at times leukemia, in chickens by means of a filter passing virus and Gordon[6] has performed experi-

ments which suggest that Hodgkin's disease may belong to the virus group. He finds that rabbits injected intra-cerebrally with emulsions of lymph glands from Hodgkin's disease patients develop a symptom complex not seen when similar emulsions prepared from lymph glands with other lesions are injected, moreover he found small bodies in lymph nodes of patients with Hodgkin's disease and in the brains of the innoculated rabbits, indistinguishable from the elementary bodies found in vaccinia and fowl-pox. Although he has not found the agent to be filterable it resembles the viruses in its behavior to heat and the antiseptics.

This by no means exhausts the subject but enough has been indicated, it is hoped, to justify the thesis that a new era in medicine has come.

W. S. THOMAS.

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†KEILTY, ROBERT A.Washington, D. C.
KELLY, FRANK L....Philadelphia, Pa.
KELLY, Wm. E....Middletown, N. Y.
KEMP, HARDY A.....Dallas, Texas
†KILDUFFE, R. A..Atlantic City, N. J.
KIM, GAY B.....Paterson, N. J.
KING, W. E.....Grosse Pointe, Mich.
KITZMILLER, K. V....Cincinnati, Ohio
KLENK, CHAS. L.....St. Louis, Mo.
KLINE, BENJAMIN S...Cleveland, Ohio
KLUGH, GEORGE F.....Atlanta, Ga.
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KONZELMANN, F. W..Philadelphia, Pa.
KOSKY, ALFRED A.Santa Monica, Calif.
KOTZ, ADAM L.....Easton, Pa.
†KRACKE, ROY R...Emory Univ., Ga.
KRAMER, G. B....Youngstown, Ohio
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†LARSON, LEONARD W.Bismarck, N. D.
†LATTIMORE, JOHN L..Topeka, Kans.
LAWRENCE, H. J.....Portland, Oregon
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†LEE, D. C.....Hot Springs, Ark.
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LEWIS, SEABORN, J..Beaumont, Texas
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LITTERER, J. H.....Nashville, Tenn.
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LOHR, OLIVER W.....Saginaw, Mich.
LOUD, N. W.....New Britain, Conn.
LOWY, O.....Newark, N. J.
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LYNCH, KENNETH M.Charleston, S. C.
LYON, M. W.....South Bend, Ind.
MACCARTY, WM. C..Rochester, Minn.
MAGATH, THOMAS B.Rochester, Minn.
MAGNUSSON, G. A.....Seattle, Wash.
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MANLOVE, C. H.....Portland, Ore.
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MCCOLL, CHARLES R..Tacoma, Wash.
MCNAMARA, F. P....Dubuque, Iowa
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MEYERS, WM. A.....Norman, Okla.
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Agram, Jugoslavia
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MORSE, PLINN F....Detroit, Michigan
MOURSUND, W. H.....Dallas, Texas
MOYER, RAY P..... Pittsburgh, Pa.
MUGRAGE, E. R.....Denver, Colo.
MUZZY, WILLIAM J....El Reno, Okla.
MYERS, R. E...Oklahoma City, Okla.
NARR, FRED C.....Kansas City, Mo.
NEAL, M. Pinson.....Columbia, Mo.
†NELSON, I. A.....Tulsa, Okla.
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NICKEL, A. C.....Bluffton, Ind.
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NORRIS, JACK C.....Atlanta, Ga.

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TRAINING SCHOOLS FOR TECHNICIANS APPROVED BY THE BOARD OF REGISTRY OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, 1933

FOREWORD

The instruction and training given to clinical laboratory student technicians in the hospital laboratories of the country still fall short of the goal set by the Board of Registry of the American Society of Clinical Pathologists which aims at a unification and standardization of the teaching program. A model curriculum is now being prepared by the Registry and will soon be available to all the training schools.

The accompanying list includes hospital laboratories which have applied for registration and have been approved by the Board as complying with the minimum requirements, the essentials of which are herewith appended.

In view of the limited number of universities and colleges of learning that are, at present, preparing their graduates for this useful vocation, it is imperative that hospital laboratories, under competent teaching direction, supplement the normal supply of adequately trained workers. It is also desirable that the fourth year of university instruction be spent wholly in a hospital laboratory.

The Board of Registry invites the directors of hospital laboratories who can comply with these requirements to register their schools under our auspices. This will help to eliminate the evil of the commercial schools which, inspired wholly by the profit end,

DATA ON TRAINING SCHOOLS FOR TECHNICIANS APPROVED BY THE BOARD OF REGISTRY OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, 1933

HOSPITAL	CITY	DIRECTOR	BED CAPACITY†	OCCUPANCY†	STAFF	STUDENTS	RATIO STAFF TO STUDENTS	LENGTH COURSE	FEE
			Class "A"*						
								months	
Geisinger Memorial	Danville, Pa.	Henry F. Hunt	180	145	6	2	3:1	32	
Henry Ford	Detroit, Mich.	Frank W. Hartman	524	390	12	4	3:1	18	
St. Joseph	Kansas City, Mo.	Emsley Johnson	230	150	5	5	1:1	15	
St. Johns[1]	Springfield, Ill.	Walter G. Bain	540	458	4	3	4:3	12	50.00
Mt. Zion	San Francisco, Calif.	Charles Weiss	172	112	10	4	2:1	18	
Mpls. General[2]	Minneapolis, Minn.	N. H. Lufkin	593	580	12	9	1:1		
Buffalo General	Buffalo, N. Y.	Byron D. Bowens	434	275	12	4	3:1	12	
			Class "B"**						
Ancker	St. Paul, Minn.	John F. Noble	1,000	703	9	6	3:2	12	
Stuart Circle	Richmond, Va.	Regina C. Beck	90	70	3	2	3:2	12	25.00
University	Omaha, Neb.	J. P. Tollman	215	178	4	5	1:1	12	1.00
St. Mary's	Duluth, Minn.	G. Berdez	260	192	4	4	1:1	15	55.00
			Class "C"***						
St. Johns	Brooklyn, N. Y.	Theo. J. Corphey	194	133	3	3	1:1	18	75.00
Mercy	Baltimore, Md.	H. T. Collenberg	260	250	7	10	1:2	12	150.00
Presbyterian	Denver, Colo.	P. C. Carson	150	99	2	2	1:1	12	.00
St. Lukes	Spokane, Wash.	R. F. E. Stier	151	94	2	2	1:1	12	10.00
Temple U.	Philadelphia, Pa.	F. W. Konzelmann	399	275	8	20	1:3	12	150.00

DATA ON TRAINING SCHOOLS FOR TECHNICIANS—*Concluded*

HOSPITAL	CITY	DIRECTOR	BED CAPACITY†	OCCUPANCY†	STAFF	STUDENTS	RATIO STAFF TO STUDENTS	LENGTH COURSE	FEE
		Class "C"***—*Concluded*							
Sacred Heart	Spokane, Wash.	M. M. Potter	300	214	5	2	2:1	12	115.00
The Grace	Detroit, Mich.	C. I. Owens	301	262	3	6	1:2	12	150.00
Mt. Sinai	Cleveland, Ohio	B. S. Klein	225	160	7	8	1:1	12	250.00
Beth Israel	Newark, N. J.	A. Yaguda	318	291	8	6	4:3	12	100.00
Wisconsin General	Madison, Wisc.	W. D. Stovall	630	604	12	12	1:1	12	25.00
Monmouth Memorial††	Long Branch, N. J.	C. A. Pons	186	159	3			12	.00
Swedish	Minneapolis, Minn.	C. R. Drake	271	184	5	10	1:2	12	125.00
Research	Kansas City, Mo.	F. C. Narr	205	141	6	5	1:1	12	.00
Jefferson	Philadelphia, Pa.	B. L. Crawford	639	524	25	5	5:1	12	100.00
The C. T. Miller	St. Paul, Minn.	Kano Ikeda	195	124	5	5	1:1	12	125.00
Uniontown	Uniontown, Pa.	H. A. Heise	200	123	3	3	1:1	24	200.00†
St. Joseph	Lexington, Ky.	E. S. Maxwell	200	126	6	3	2:1	12	150.00
St. Joseph	Louisville, Ky.	H. W. Weeter	300	152	4	2	2:1	12	120.00
Mt. Sinai	Chicago, Ill.	I. Davidson	160	106	5	3	2:1	18	100.00
L. Y. P. Montgomery	Battle Creek, Mich.	A. A. Humphrey	180	84	3	4	1:1	12	Variable
U. of Penn.††	Philadelphia, Pa.	H. Fox	450		14	4	3:1	12†	150 00 and 50.00
Mt. Sinai	Philadelphia, Pa.	D. R. Meranze	316	214	8	6	1:1	18	125.00
Mercy	Bay City, Mich.	W. G. Gamble	144	63	3	2	2:1	12	150.00

Class "X" ⁂ §

INSTITUTION	CITY	DIRECTOR	COURSE	LENGTH COURSE	HOSPITAL CONNECTION
				years	
Emory University	Emory University, Ga.	Roy R. Kracke, M.D.	Graduate	1–1¼	?
Mich. State Coll. of Agriculture	East Lansing, Mich.	Ward Giltner, Ph.D.	College	4	?
North Carolina Coll. for Women	Greensboro, N. C.	Lila B. Love, M.S.	College	4	?
U. of Denver	Denver, Col.	E. A. Engle, Ph.D.	College	4	Yes
Simmons College	Boston, Mass.	C. M. Hilliard, A.B.	College	4	Yes
U. of Kentucky	Lexington, Ky.	M. Scherago, Ph.D.	College	4	Yes
Ohio University	Athens, Ohio	F. H. Krecker, Ph.D.	College	4	Yes

* Class A entrance requirements: Four years of college work, including chemistry and biology.

** Class B entrance requirements: Two years of college work, including chemistry and biology.

*** Class C entrance requirements: One year of college work, including chemistry and biology.

⁂ Class X entrance requirement: High school certificate, except that Emory University requires an A.B. or B.S. degree. The course is a regular university or college course in each school listed under this heading.

† Bed capacity and occupancy as published in the Hospital Number, March 25, 1933 issue of the Journal of the American Medical Association.

†† Training school temporarily discontinued.

[1] Graduate nurses admitted in lieu of other requirements for admission.

[2] Fourth year students in Medical Technology course in U. of Minnesota admitted.

§ Affiliation with a general hospital to have the students spend their last academic year in the service of its clinical laboratories is considered essential.

turn out inadequately trained technicians with resultant loss of time and money to the duped students and cause harm to the patient if these ill prepared workers secure a position from an unwary employer.

With the encouragement and indorsement given the Registry by the American Medical Association, the American College of Surgeons and the American Hospital Association, it is hoped that in a few years it will be possible to have the training of technicians on a stable and scientific basis.

ESSENTIALS FOR APPROVED HOSPITAL LABORATORY TRAINING COURSE FOR TECHNICIANS

(Apprenticeship Plan)

(1) The Director must be a graduate in medicine and a clinical pathologist of recognized standing.

(2) The technical staff shall consist of a sufficient number of registered laboratory technicians, capable of teaching, demonstrating and directing the work of the individual student.

(3) The yearly enrollment shall not exceed more than two students to each member of the teaching staff.

(4) The hospital shall have a bed capacity for not less than 100 patients and an average occupancy of eighty-five per day.

(5) The minimum educational pre-requisites shall be (a) high school graduation, and (b) the credits of one year college work including chemistry and biology. Graduation from a recognized nursing school may be considered an equivalent, provided the applicant has a high school diploma.

(6) The minimum length of the course for training shall be not less than twelve months, consisting of a rotating or departmentalized service with the minimum 300 laboratory and twenty didactic hours in each two month period of service.

(7) The instruction shall include (a) didactic period, (b) text assignment, (c) quiz hour, (d) periodic written examination, (e) practical demonstration, (f) practice period and performance of tests under supervision.

(8) There shall be adequate equipment and space, as well as a sufficient number and variety of specimens, to meet the added requirements of training and practice of the students.

(9) Commercial advertising is considered unethical.

News and Notices

Through an oversight, the name of Dr. Zera E. Bolin was omitted from the Public Relations Committee in the report which appeared in the September issue of the Journal.

The following committee has been appointed: Committee on Investigation of Molds—Dr. F. M. Johns and Dr. W. D. Stovall.

Dr. A. V. St. George is chairman of the Committee on Necropsies instead of Dr. F. E. Sondern as announced in the last issue of the JOURNAL.

BOOK REVIEWS

Textbook of Neuropathology. By Arthur Weil. Pp. 330, 1933, Philadelphia, Lea and Febiger, $5.00.

Neuropathology has developed so rapidly and extensively that it is almost impossible to include in one volume a complete review, or even a summary, of the subject. As must be expected, in a volume of the size of this one, some diseases are omitted and others are treated rather briefly, but practically all of the more common diseases and some of the rarer diseases are included. The chapters on autolysis, and on chemical and metabolic changes are useful, and the tables accompanying them are good, and should be valuable to students of the subject. Included in the book are twenty-nine tables, collected from various authorities, and most of these will prove of value to pathologists and neurologists. The volume is well illustrated, which adds materially to the value of the book. The majority of the numerous illustrations, both gross and microscopic, are original.

—J. W. Kernohan.

Histopathology of the Peripheral and Central Nervous Systems. By George B. Hassin. Pp. 479, 1933, Baltimore, The William Wood and Company, $6.00.

This book represents the results of twenty years' experience in neuropathology, and is based on wide knowledge of the literature on the subject. Practically all diseases of the nervous systems are considered, and various views are presented, accompanied by the author's own views and observations. Most of the teaching is orthodox; that dealing with the formation, circulation, and absorption of the cerebrospinal fluid, however, is original and heterodox, but cannot be lightly disregarded. The chapters on inflammatory diseases are especially complete, as are those on the degenerative diseases, both of the brain and spinal cord. The type is clear; the volume is profusely illustrated with excel-

lent photographs and photomicrographs, and with some drawings. The extensive and complicated subject is presented in an excellent manner. The book can be recommended to students of neuropathology, as well as to neurologists and general pathologists.

—J. W. KERNOHAN.

American Journal of Clinical Pathology

Manuscripts and books for review should be sent to Dr. Thomas B. Magath, Mayo Clinic, Rochester, Minnesota. Manuscripts must be typewritten and all figures and tables should be in such form as to be ready for the printer. The expense for a limited number of cuts can be borne by the Society; expense for cuts in excess of this number will have to be defrayed by the author. The nomenclature for species of bacteria will be that given in Bergey's "Manual of Determinative Bacteriology." Bibliographic references will be limited to the papers actually referred to in the text. Such citations must be arranged in alphabetic sequence and made in the following form: author's name followed by initials, title, journal, volume, inclusive pages, date.

(Examples) Kolmer, J. A.: Toxin production by *Spirochaeta pallida*. Arch. Derm. and Syph., 20: 189–190. 1929.

McFarland, Joseph: A text book upon the pathogenic bacteria and protozoa for students of medicine and physicians. Philadelphia and London: W. B. Saunders Company, 1919, pp. 858.

Twenty-five reprints, without covers, of articles will be furnished gratis to contributors when ordered in advance. A table showing cost of additional reprints, with an order slip, is sent with proof.

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